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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF POTENTIAL HYPOTENSIVE AGENTS

by

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A THESIS
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ABSTRACT

A series of 3-aryl- and 3-arylalkyl-aminopropionates and the corresponding aminohydroxamic acids were synthesized and screened for hypotensive activity using anaesthetized rats. The results are interpreted in terms of the structural features affecting activity in these compounds.

The mechanisms of action of all active compounds were investigated. Several of these compounds were found to act *via* ganglionic blockade.

All aminohydroxamic acids and amino-esters in this study were screened in mice for potential protection against poisoning by di-*iso*propylfluorophosphonate (DFP). None of these compounds were effective in protecting mice against organophosphate poisoning.

The aminohydroxamic acids were synthesized by the interaction of various amines and α,β-unsaturated esters or 3-bromo-esters, and subsequent treatment of the amino-ester intermediates with hydroxylamine hydrochloride.

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ABBREVIATIONS

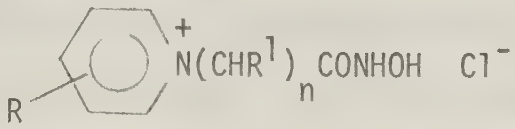
The following abbreviations have been used throughout the text:

ACh	Acetylcholine
AChE	Acetylcholinesterase
DFP	Di-*iso*propylfluorophosphonate
Nmr	Nuclear magnetic resonance
Ir	Infra-red
TMS	Tetramethylsilane
ip	Intraperitoneal
sc	Subcutaneous

CHAPTER 1

INTRODUCTION

Coe (1959) synthesized quaternary pyridinium hydroxamic acids of general structure (1)



(1)

(where R is a hydrogen atom, an alkyl group or substituted alkyl group, and R^1 is a hydrogen atom ($n = 1$ or 2) or a methyl group ($n = 1$)) and found them effective in the prophylaxis and treatment of organophosphate poisoning.

This report was of great interest to Coutts et al (1969), who extended their work in the field of hydroxamic acids to include synthesis and pharmacological evaluation of compounds of general structure (2) where

$$R^1R^2\overset{+}{N}HCH_2CHR^3CONHOH\quad Cl^-$$

(2)

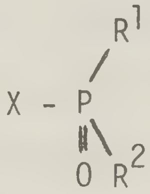
R^1 and R^2 were alkyl groups or cyclic systems, and R^3 was a methyl group or hydrogen atom. Although initially prepared as potential protectives or antidotes in organophosphate poisoning, it was soon learned that these compounds possessed hypotensive activity.

Subsequent investigations (including the present study) by Coutts and his co-workers have stressed the search for new hypotensive agents chemically related to those of general structure (2). Nevertheless, most of the compounds synthesized by this group

have been tested for their efficacy in combating organophosphate poisoning, and a brief discussion of the latter topic follows.

The widespread use of organophosphorous compounds (organophosphates) as insecticides and their potential use in chemical warfare necessitates the development of effective antidotes for organophosphate poisoning.

Although thousands of organophosphates have been synthesized, all share a common biological action, cholinesterase inhibition. They are usually pentavalent phosphorous compounds of general structure (3)



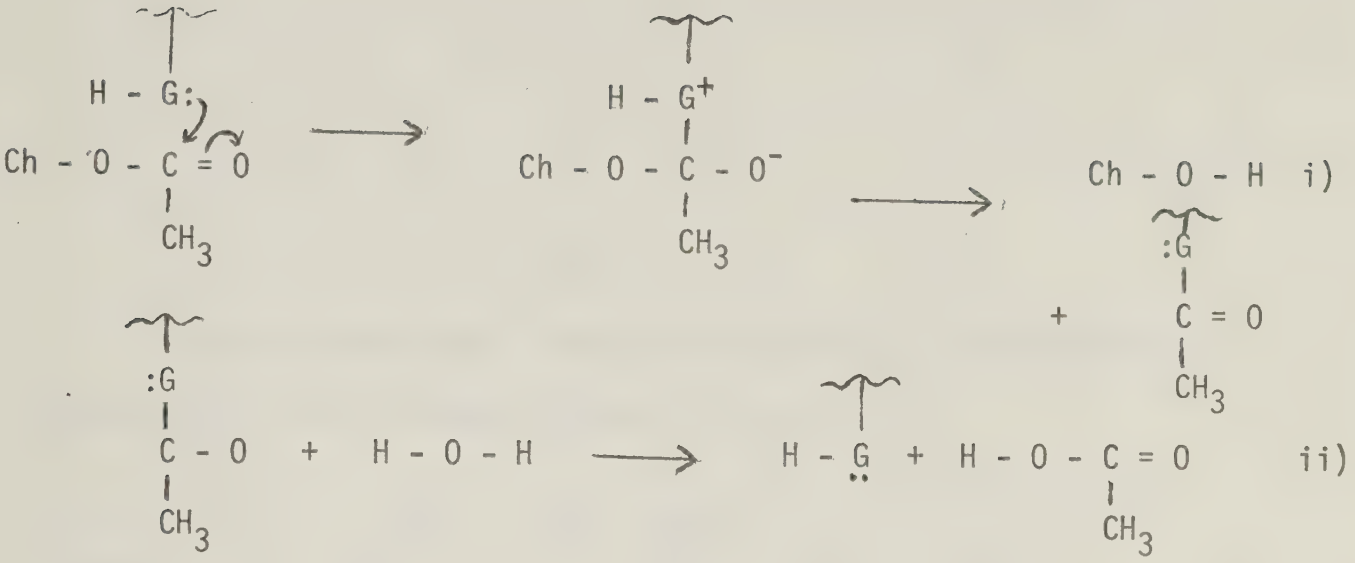
(3)

where R^1 is an alkoxy group, R^2 is an alkoxy, alkyl or dialkylamido group, and X is a halogen, cyanide, phenoxy, or di-substituted phosphoryloxy group. The organophosphates may be further classified on the basis of their physical properties. Those which are potent anticholinesterases and are also volatile are suitable for use as 'nerve gases'. Examples of the latter compounds include sarin (*iso*propylmethylphosphonofluoridate)(3,$R^1 = CH_3$, $R^2 = (CH_3)_2CHO$ and $X = F$) and tabun (ethyl-N,N-dimethylphosphoramidocyanidate) (3, $R^1 = (CH_3)_2N$, $R^2 = OC_2H_5$ and $X = CN$). Those organophosphates which are weak anticholinesterases (but are highly active when

metabolized by insects) and are non-volatile are useful as insecticides. A well-known example of the latter group of organophosphates is malathion (O,O-dimethyl-S-(1,2-dicarbethoxyethyl)-phosphorodithionate). This compound is rapidly metabolized in mammals to a non-toxic mono-ester, whereas in insects, the main metabolic product is malaoxon, a potent anticholinesterase.

An agent which protects against organophosphate poisoning must prevent the association of AChE and organophosphate. A knowledge of the interaction between ACh and AChE during the hydrolysis of ACh by the enzyme is useful in the development of such an agent. Various reports (Wilson and Bergman, (1950); Bergman et al, (1950); Wilson et al, (1950)) have indicated that the groups in the ACh molecule which are most likely to be bound to the AChE surface are the cationic head and the ester group. Those parts of the AChE surface which may bind the cationic head and ester group of ACh have been designated the 'anionic' and 'esteratic' sites, respectively.

Wilson et al (1950) have suggested that the hydrolysis of ACh by AChE proceeds via a mechanism wherein a basic group in the AChE esteratic site forms a covalent bond with the carboxyl carbon atom of AChE:

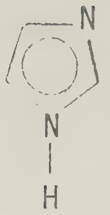


$H - \underset{\cdot\cdot}{G}$ is the part of the esteratic site of AChE containing a basic group represented by a lone pair of electrons and an acidic group, represented by a hydrogen atom. Ch is $CH_2CH_2\overset{+}{N}(CH_3)_3$.

Krupka and Laidler (1961) suggest that the oxygen atom of the hydroxyl group of a serine residue forms a covalent bond during the hydrolysis (*i.e.*, H - G: is $H - \underset{\cdot\cdot}{O}:$).

Another worker (Triggle, 1965) believes that four groups in two sites of the enzyme are involved in binding by AChE: the anionic site, an acidic group, a basic group and a serine residue of the esteratic site. Ariens (1962) suspects that the anionic site which binds the trimethylammonium group of ACh is a phosphoric acid group, and that the esteratic site binds and reacts with the acetyl group of the substrate.

Krupka and Laidler (1961) suggest that the basic group may have an imidazole (4) nucleus.



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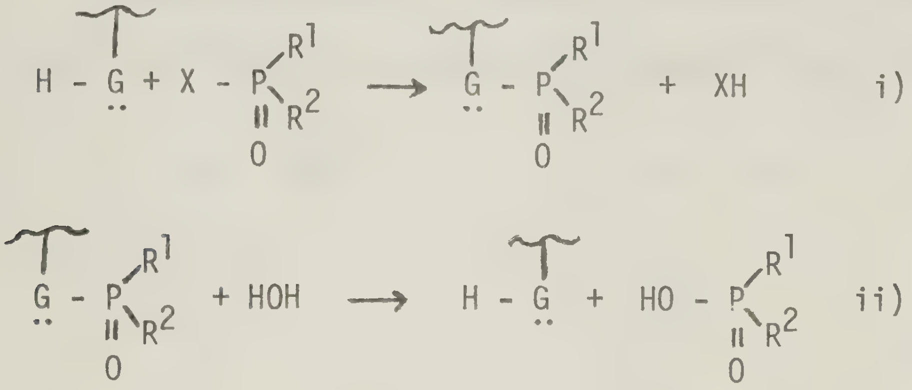
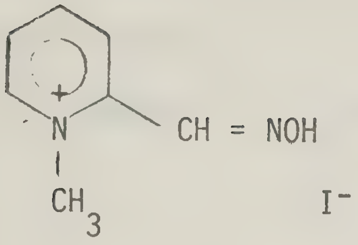
The 'irreversible' inhibition of AChE by organophosphates has been outlined as follows (Wilson, (1951)):



Since phosphorylated AChE is very stable, hydrolytic step (ii) is very slow.

Substances which destroy the organophosphate-AChE complex and return normal AChE activity are termed 'reactivators'. Wilson (1951) discovered that choline and hydroxylamine reactivated phosphorylated AChE. This discovery led to the development of rapid reactivators such as various oximes and hydroxamic acids. The best known examples of oxime reactivators are pralidoxime iodide (2-pyridine aldoxime methiodide) (5), and N,N-trimethylene bis (4-hydroxyiminomethylpyridinium) dibromide (6).



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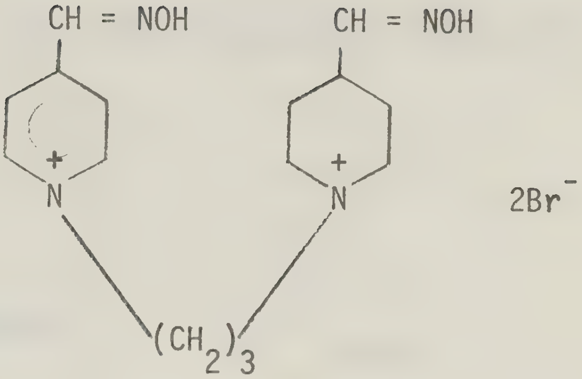
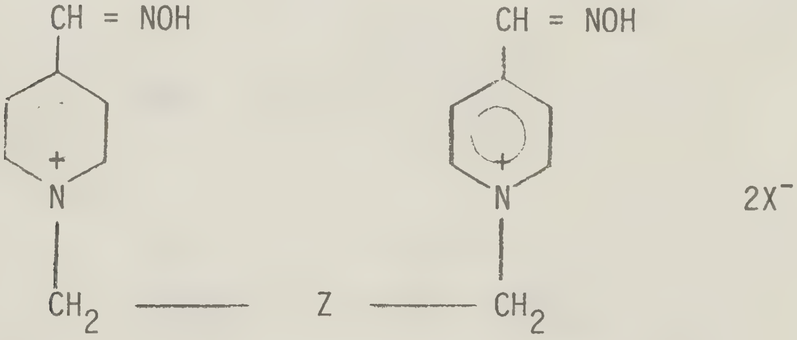


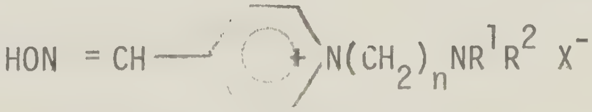
(6)

Oxime reactivators of general structure (7) where Z is O, S, SO, or SO_2 have been prepared by Hauschild et al (1965).



(7)

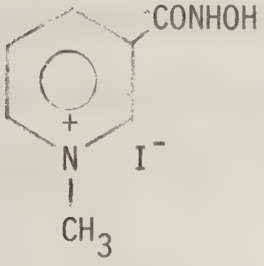
Ashani and Cohen (1967) found that in a series of oximes of general structure (8), 4-hydroxyiminomethyl-1-(3(N,N-dimethylamino)-n-propyl)-pyridinium chloride provided good protection against organophosphate poisoning.



(8)

Hydroxamic acid reactivators (less potent than oxime reactivators) include nicotinohydroxamic acid methiodide (9),

pyrimidine-2-hydroxamic acid (10), and picolinohydroxamic acid (11) (Hobbiger, (1963)).



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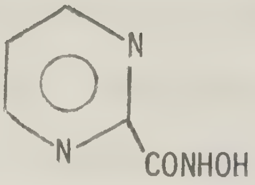

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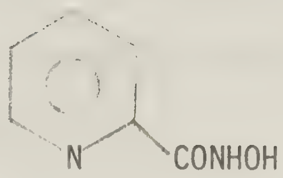
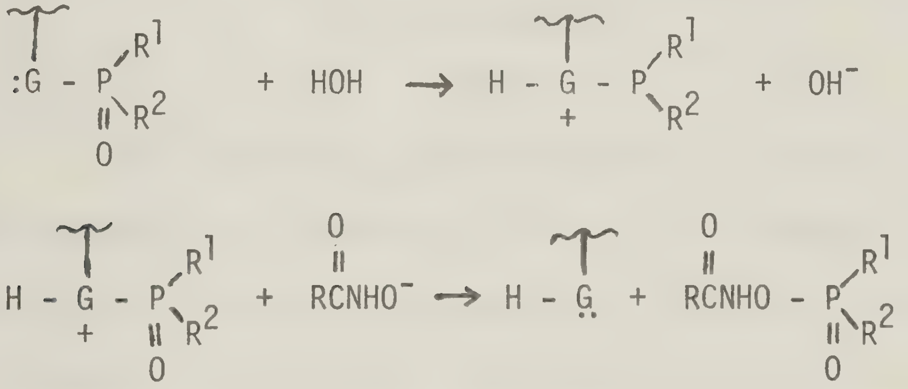


(11)

The reactivation of phosphorylated AChE by hydroxamic acids has been represented as follows (Hobbiger, (1963)):



Gilbert et al (1961) prepared a series of hydroxamates of general structure (12) envisaged as potential reactivators of phosphorylated AChE. These compounds were found ineffective as reactivators, despite their similarity to the ACh molecule.

$$(CH_3)_3\overset{+}{N}(CH_2)_nCONHOH\ Cl^- \qquad n = 1 \text{ to } 3$$

(12)

As previously mentioned, Coutts et al (1969) attempted to prepare reactivators structurally resembling those reported by

Coe (1959). Coutts and his co-workers first synthesized a series of methyl 3-aminopropionate hydrochlorides of general structure (13) where R^1 was a substituted or unsubstituted piperidino, homopiperidino, morpholino, piperazino, or benzylamino group and R^2 was a methyl group or hydrogen atom. The latter

$$R^1CH_2CHR^2COOCH_3\ HCl$$

(13)

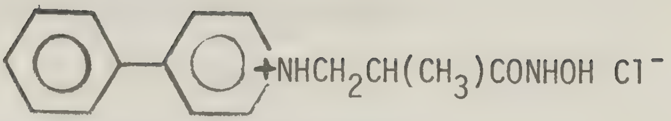
compounds were then converted to the corresponding 3-aminopropionohydroxamic acid hydrochlorides of general structure (14).

$$R^1CH_2CHR^2CONHOH\ HCl$$

(14)

Intraperitoneal administration of large doses of the aminohydroxamic acid hydrochlorides to rats produced disorientation, neurological effects (e.g. dyspnea) and CNS stimulation followed by depression. These observations led Coutts and his co-workers to evaluate the effect of both the 3-aminopropionohydroxamic acids and 3-aminopropionates on the carotid arterial blood pressure of anaesthetized cats. All hydroxamic acids and most of the esters caused a fall in blood pressure, the hydroxamates having a longer duration of action.

2-Methyl-3-(4-phenylpiperidino)propionohydroxamic acid hydrochloride (15) was pharmacologically the most interesting of the compounds tested in the previous study, producing a marked and prolonged fall in arterial blood pressure.

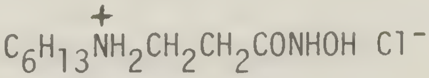


(15)

Subsequent detailed pharmacological investigation of the hypotensive action of compound (15) by Midha et al (1970) suggested that it may induce hypotension by sympathetic ganglion blockade. The latter compound was found to be as effective a hypotensive agent as pentolinium tartrate on a molar basis.

Recognizing that the nature of the amino moiety R^1 in compounds of general structures (13) and (14) was important in determining the magnitude and duration of the fall in blood pressure observed, Coutts et al (1971) extended their investigations to include compounds wherein R^1 was an alkyl or dialkylamino group, in addition to compounds wherein R^1 was a piperidino or morpholino group. R^2 remained a methyl group or a hydrogen atom. The compounds were evaluated for their effect on the blood pressure of anaesthetized cats.

Certain members of this new series of compounds produced prolonged falls in blood pressure, namely, 3-hexylaminopropionohydroxamic acid hydrochloride (16), methyl 2-methyl-3-dimethylaminopropionate hydrochloride (17), and 2-methyl-3-phenethylaminopropionohydroxamic acid hydrochloride (18).

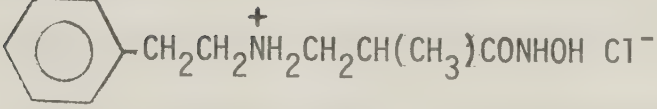


$$C_6H_{13}\overset{+}{N}H_2CH_2CH_2CONHOH\ Cl^-$$

(16)

$$(CH_3)_2\overset{+}{N}HCH_2CH(CH_3)NHOH\ Cl^-$$

(17)



(18)

None of the latter three compounds were as active as compound (15) reported by Midha et al (1970).

Several aminopropionic acids of general structure (19) where R was piperidino or substituted piperidino, homopiperidino, or ethylamino, and R^1 was a hydrogen atom or methyl group, were also tested and found to have no effect on the blood pressure of anaesthetized rats.

$$RCH_2CHR^1COOH$$

(19)

The synthesis and pharmacological evaluation of amino-esters and their corresponding hydroxamic acids has been continued by Coutts and his co-workers.

Selley (1971) has synthesized a series of amino-ester and amino-hydroxamic acid hydrochlorides of general structures (20) and (21) wherein R^1 was an alkyl group, an alicyclic ring, or an allyl group,

$$R^1\overset{+}{N}H_2CH_2CHR^2COOCH_3\ Cl^-$$

(20)

$$R^1\overset{+}{N}H_2CH_2CHR^2CONHOH\ Cl^-$$

(21)

and has reported the pharmacological evaluation of many of these amino-esters and all of the hydroxamates for their effect on the arterial blood pressure of anaesthetized rats.

Selley concluded that for aminohydroxamic acids of general structure (21) the nature of R^1 is highly critical, and found that, depending upon the nature of R^1, the introduction of a methyl group (R^2) could inactivate the compound. It was also found that for compounds where R^1 was a straight chain alkyl function, and R^2 was a hydrogen atom, n-hexyl offered the greatest hypotensive activity.

The same author has also reported that one of the amino-esters synthesized in his study, methyl 2-methyl-3-octylamino-propionate hydrochloride (22) had a marked hypotensive effect, lowering blood pressure by sympathetic and parasympathetic ganglion blockade.

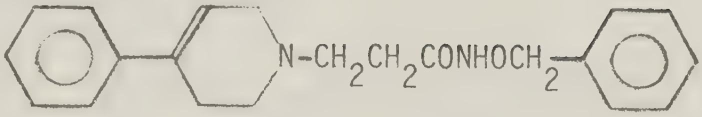
$$C_8H_{17}\underset{+}{N}H_2\ CH_2CH(CH_3)COOCH_3\ Cl^-$$

(22)

All active compounds in the latter study had short durations of action at doses of 4 mg/kg. (None of the hydroxamates synthesized were found effective against organophosphate poisoning.)

Aralkoxyamides of 4-phenyl-1,2,5,6-tetrahydropyridino-alkanoic acid such as (23) has been suggested (Biel, 1965) for use in the treatment of hypertension and peripheral vascular disease.

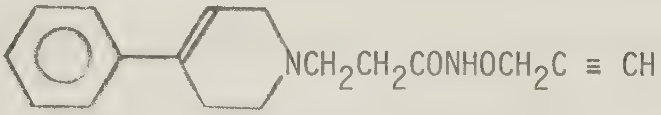
These compounds reportedly



(23)

produce a potent and long lasting fall in blood pressure with a minimum of side effects as their mechanism of action does not involve blockade of peripheral and autonomic ganglia.

The closely related N-alkynyl and N-alkynyloxy derivatives (24) have analgesic activity (Biel and Hopps, 1965).



(24)

In summary, little has been published concerning the synthesis and pharmacological evaluation of aminohydroxamic acids similar to those of general structure (2).

In contrast, a considerable amount of information regarding the chemistry and pharmacology of amino-esters similar to those of general structural type (20) exists in the literature. It is now convenient to discuss the pharmacology of these compounds, postponing an examination of their chemistry until later.

Aliphatic 3-amino-esters and related compounds have a wide range of biological activities.

Halverstadt et al (1959) have investigated the synthesis and hypotensive activity of a series of 2-ammonioalkyl 3-ammonioal-

kanoate salts derived from general structure (25) where R^1 ...R^6 are various alkyl substituents

$$R^1R^2R^3\overset{+}{N}CH_2CH_2COOCH_2CH_2NR^4R^5R^6\ 2X^-$$

(25)

or heterocycles. A number of these diammonio-esters exhibited marked hypotensive activity via ganglion blockade.

Pacheo et al (1962) have screened a number of amino-esters of general formula (26) (in which R^1 was a piperidino, pyrrolidino or morpholino group, R^2 was usually a phenyl group, and R^3 was a methyl or higher group) for antispasmodic activity. Butyl 3-phenyl-3-piperidylpropionate hydrochloride

$$R^1CHR^2CH_2COOR^3\ \ HCl$$

(26)

and isobutyl 3-phenyl-3-pyrrolidylpropionate hydrochloride showed low toxicity and high activity.

Adamson (1954) has reported that compounds of general structure (27) where R^1 is methyl, R^2 is methyl or ethyl, and R^3 is methyl or propyl have strong analgesic properties.

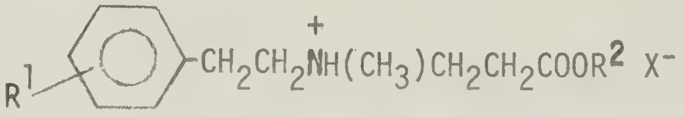
$$R^1R^2NCHR^3CH_2COOCH_3$$

(27)

In a series of papers, Baltzly and Phillips and their co-workers have screened amino-esters closely related to those

prepared in the present study for oxytocic activity.

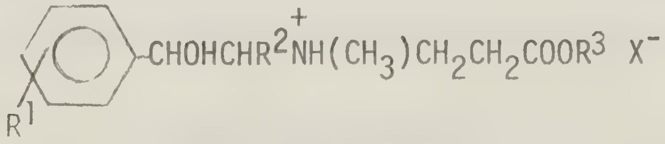
Baltzly _et al_ (1949) initially prepared a series of compounds of general structure (28) where R^2 was a methyl or ethyl group, R^1 was an alkoxy or phenoxy substituent and X was a Cl or Br atom. Of the various



(28)

derivatives tested for oxytocic activity (in analogy to lysergic acid) those with two alkoxy substituents on the phenyl ring were most active (5 - 10% of that shown by ergonovine per unit weight). Phenolic derivatives were inactive.

Baltzly and Phillips (1949[a]) extended their investigation to include compounds of structural formula (29), which featured a hydroxylated side-chain. These compounds proved to be comparable in oxytocic activity to those

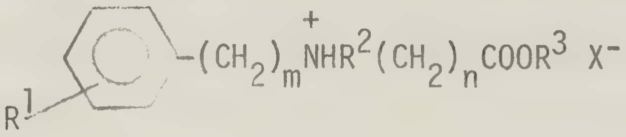


(29)

of general structure (28) when they possessed two alkoxy substituents (R^1), R^2 being a hydrogen atom, and R^3 an ethyl or methyl group.

Monoalkoxy and phenolic compounds showed activity comparable to the previous dialkoxy compounds only when R^2 was a methyl group.

Baltzly and Phillips (1949[b]) next studied the effect of chain length on oxytocic activity in compounds of general formula (30). Compounds where R^1 was



(30)

3,4-$(CH_3O)_2$, m equalled 2, R^2 was a methyl group, n equalled 1, R^3 was an ethyl and X was Cl and similar compounds with unsubstituted phenyl rings were found inactive as oxytocics. However, compounds where n equalled 2 were active. It was concluded that at least two carbons must intervene between the amino and carbalkoxyl groups. However, an increase in n over two units generally produced no increase in activity, and decreased activity in some cases.

Phillips (1950) continued the search for oxytocic 3-aminopropionates, synthesizing compounds of general structure (31).

$$R^1R^2\overset{+}{N}HCH_2CH_2COOCH_3\ Cl^-$$

(31)

where (a) R^1 and R^2 were both n-alkyl groups, (b) R^1 was methyl, benzyl, 4-methoxyphenethyl or 3,4-dimethoxyphenethyl and R^2 was

2-carbomethoxyethyl or (c) R^1R^2N was a saturated heterocyclic ring.

None of these compounds were oxytocics, but were generally of low toxicity.

Another group, Horii et al (1962) has evaluated 3-amino-ester derivatives of general structure (32) (where R^1 was a variety of aliphatic or aromatic groups, and R^2 and R^3 were methyl groups or hydrogen atoms) for oxytocic activity. Methyl 2-methyl-3-[2-(3-indolylethyl)]propionate

$$R^1R^2\overset{+}{N}HCH_2CHR^3COOCH_3\ Cl^-$$

(32)

hydrochloride showed the highest oxytocic activity of all compounds synthesized in this study.

A compound resembling general structure (2), the reversed carboxyl analogue (33) of ACh, was synthesized by Bass et al (1950) and found equipotent with ACh on guinea-pig ileum.

$$(CH_3)_3\overset{+}{N}CH_2CH_2COOCH_3\ I^-$$

(33)

Schueler and Keasling (1951) have reported that the reversed carboxyl analogues of acetyl-α-methylcholine and acetyl-β-methylcholine, (34) and (35), respectively possessed less than one ten-thousandth of the muscarinic activity of their respective ACh analogues.

$$(CH_3)_3\overset{+}{N}CH(CH_3)CH_2COOCH_3\ I^-$$

(34)

$$(CH_3)_3\overset{+}{N}CH_2CH(CH_3)COOCH_3\ I^-$$

(35)

A comparison of the differences in reported relative activities of compounds (33), (34) and (35) prompted Biggs *et al* (1971) to synthesize and re-examine the muscarinic and nicotinic activities of all three esters. It was found that compounds (34) and (35) exhibited significant muscarinic action, while compounds (33) and (34) possessed nicotinic action. Compounds (34) and (35) were found to be more potent parasympathomimetics than previously reported.

Matkovics *et al* (1961 and 1962) and Porszasz *et al* (1961) synthesized and evaluated amino-esters of general structure (36) where R^1 was a

$$R^1(CH_2)_nCOOR^2 \qquad n = 1 \text{ or } 2$$

(36)

piperidino, pyrrolidino or morpholino group and R^2 was a methyl, ethyl, butyl or benzyl group. Both tertiary esters and those with lower aliphatic substituents exhibited nicotinic action, while those with higher molecular weight substituents or aromatic

substituents reportedly produced ganglion blockade.

Amino-esters of structural type (36) were also studied by Barrass *et al* (1968). These workers found that these compounds (where R^1 was a

$$R^1(CH_2)_nCOOR^2 \qquad n = 1 \text{ to } 4$$

(36)

dimethylamino or pyrrolidino group and R^2 was a methyl or ethyl group) were predominantly nicotinic, and were more active when quaternized.

The same workers re-evaluated the reversed carboxyl analogue of ACh (33)

$$(CH_3)_3\overset{+}{N}CH_2CH_2COOCH_3\ I^-$$

(33)

and assigned it the 0.16 the activity of ACh (compared with the equipotent rating assigned by Bass *et al* (1950)).

In a subsequent report, Brimblecombe and Sutton (1968) investigated the effects of various quaternary amino-esters (such as methyl 3-dimethylaminopropionate methiodide and ethyl 3-dimethylaminopropionate methiodide) which exhibited both muscarinic and nicotinic activity on the superior cervical ganglion of the cat. They concluded that these compounds produce their effects by interaction with post synaptic receptors in the ganglion.

It should be noted that the evaluation of 3-aminopropionic acid derivatives has by no means been confined to the field of medicinal chemistry. Various 3-aminopropionates have been reported active against the tobacco mosaic virus (Darlington, 1960). The acid itself has been claimed (Pollack, 1943) to be a growth stimulant for some bacteria and yeasts. Various 3-aminopropionates have been found active as plant growth regulators (Gerjovich and Harrison, 1966). Finally, various of these esters have been reported useful (Humphreys _et al_, 1964) as hardening agents for epoxide resins.

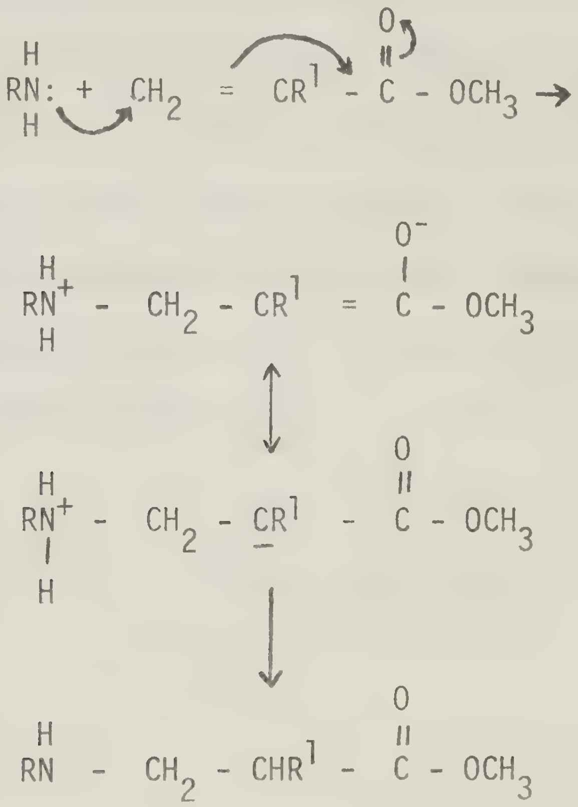
It is now convenient to discuss briefly the synthesis of the amino-esters prepared in this study. Many of the amino-esters of general structure (38) reported in the literature have been obtained by the addition of the appropriate amine to a substituted or unsubstituted ester of an α,β-unsaturated acid (39).

$$\underset{}{R^1R^2NH} \quad + \quad \underset{(39)}{CHR^3 = CR^4COOR^5} \longrightarrow \underset{(38)}{R^1R^2NHCHR^3CHR^4COOR^5}$$

(Due to the large volume of literature in this area, only selected studies can be discussed.)

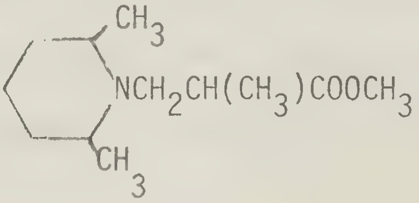
The preparation of both amino-esters and amino-di-esters via the Michael addition of amines and α,β-unsaturated esters is generally facile, using lower aliphatic esters of acrylic, metha-

crylic and crotonic acid. However, it would be expected from an examination of the mechanism of the Michael addition (shown as postulated by Pfau in 1967), that steric effects are important considerations in this reaction's use.



A report by Luskin *et al* (1956) illustrates that the size and branching pattern of alkyl groups of the amine affect product yield. The yields obtained in the addition of *n*-butylamine, *tert*-butylamine, and 1,1,3,3,-tetramethylbutylamine to methyl methacrylate were 58% methyl 2-methyl-3-*n*-butylaminopropionate, 26% methyl 2-methyl-3-*tert*-butylaminopropionate and 14% methyl 2-methyl-3-(1,1,3,3-tetramethylbutylamino)propionate, respectively.

Coutts et al (1969; 1971) observed that di-n-propylamine reacted slowly with methyl methacrylate. Attempts to add di-isopropylamine to methyl methacrylate in boiling methanol or n-butanol over extended periods of time were failures. These findings are in agreement with those of Luskin et al (1956), but were in contrast to those of Suminov (1967) who reported the successful preparation of methyl 2-methyl-3-di-isopropylaminopropionate in 33% yield with a reaction time of 24 hours. The latter investigator has also reported the preparation of methyl 2-methyl-3-(2,6-dimethylpiperidino)propionate (39) in 70% yield from 2,6-dimethylpiperidine and methyl methacrylate.



(39)

Coutts et al (1971) were unable to repeat the latter synthesis, concluding that steric hindrance prevented reaction at the nitrogen atom that had two methyl groups on adjacent carbon atoms.

The reactivity of α,β-unsaturated esters towards amines varies according to the degree of alkyl substitution on the ester. Howton (1945) reported that acrylates are more reactive than methacrylates towards primary and secondary amines. Aniline

combined with methyl acrylate to give methyl 3-anilinopropionate, while no product was obtained with methyl methacrylate. Pollard et al (1953) found that methacrylates failed to react with 1-phenylpiperazine or 1-(2-methylphenyl)-piperazine.

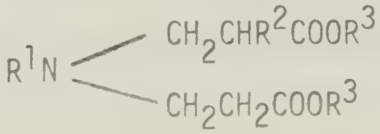
Phillipi and Galter (1929) have made a detailed study of the action of ammonia and amines upon esters of unsaturated acids. They reported that ethyl crotonate and methylamine gave mainly 3-methylaminobutyric methylamide and only a small amount of ethyl 3-methylaminobutyrate. With piperidine, only ethyl 3-piperidinobutyrate was obtained. Ethyl 2-ethylacrylate did not combine with either liquid ammonia or with methylamine. The addition of ethyl 3-dimethylacrylate and ammonia gave 60% yield of addition product, but not amides, whereas methylamine and the same ester gave methylamide. Piperidine failed to combine with ethyl 2-ethylacrylate.

Morsch (1932; 1933) in a series of three reports described the action of ammonia, methylamine, and diethylamine on ethyl crotonate, ethyl methacrylate and ethyl acrylate, respectively. This work is interesting, since it established that the products formed in the addition reactions are dependent upon: (a) relative amounts of the reactants, (b) reaction time, (c) reaction temperature (d) use of a solvent. For example, while 3.6 moles methylamine reacted with 1 mol ethyl crotonate, giving 47% ethyl 3-methyl-3-methylaminopropionate after 3 days reaction time, 1.1 mol of

methylamine and 1 mol of ethyl crotonate gave 86% ester after 8 hours reaction time (at 65^0). Ethyl crotonate (20 g) added to a saturated ethanolic solution of ammonia at room temperature and allowed to stand 5, 17, and 33 days gave ethyl 3-methyl-3-aminopropionate in 41, 55 and 50% yields, respectively. Crotonic acid (8 g) and diethylamine (15 g) in 50 ml of ethanol gave after 8 days 11% ethyl 3-diethylaminobutyrate, but if no solvent was used, the only product was crotondiethylamide.

Bieber (1950; 1954) has studied the addition of methyl methacrylate to amines and reported that the ease of reaction is piperidine>diethylamine>aniline>N-ethylaniline. Piperidine combined with methyl methacrylate in 90% yield at room temperature to give methyl 2-methyl-3-piperidinopropionate. Diethylamine added reversibly to methyl methacrylate at 120^0 to give methyl 2-methyl-3-diethylaminopropionate in 66% yield. Aniline added to methyl methacrylate only at 200^0 in a sealed tube, giving 6% methyl 2-methyl-3-anilinopropionate. N-ethylaniline gave only a small amount of impure N-ethylmethacrylamide when reacted with methacrylate. Bieber has concluded that the ease of addition depends upon both the basicity and steric hindrance of the amine molecule.

The synthesis of amino-di-esters of general formula (40) where R^1



$$R^1N\begin{cases} CH_2CHR^2COOR^3 \\ CH_2CH_2COOR^3 \end{cases}$$

(40)

is an arylalkyl group of alkyl group, and R^2 and R^3 are alkyl groups, has been described in the literature.

Howton (1945) synthesized an amino-di-ester of general structure (40) (where R^1, R^2, and R^3 were methyl groups) as an intermediate in the preparation of 1,3-dimethylpiperidin-4-one. The reaction between methyl methacrylate and methyl 3-methylaminopropionate was found not to afford a preparative route to N-(2-carbomethoxyethyl)-N-(2-carbomethoxypropyl)methylamine.

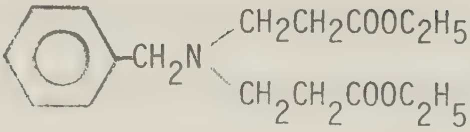
Instead, methyl acrylate was added to methyl 2-methyl-3-methylaminopropionate to give the desired product. Aniline reacted with methyl acrylate to give methyl 3-anilinopropionate, but no di-ester.

McElvain and Stork (1946) have concluded that the addition of ammonia to methyl acrylate and the transformations of the resulting ester of 3-aminopropionic acid (41) into the secondary (42) and tertiary (43) amino-esters are a series of reversible reactions.

$$\begin{tikzcd}
\underset{(43)}{N(CH_2CH_2COOC_2H_5)_3} \arrow[r, shift left] & \underset{(42)}{HN(CH_2CH_2COOC_2H_5)_2} + CH_2 = CHCOOC_2H_5 \arrow[l, shift left] \arrow[d, shift left] \\
NH_3 + CH_2 = CHCOOC_2H_5 \arrow[r, shift left] & \underset{(41)}{H_2NCH_2CH_2COOC_2H_5} + CH_2 = CHCOOC_2H_5 \arrow[u, shift left] \arrow[l, shift left]
\end{tikzcd}$$

Morosawa (1958) described the preparation of N,N-di-(2-carbethoxyethyl)benzylamine (44) from ethyl acrylate and

benzylamine. He reached the important



(44)

conclusion that (44) is decomposed by heating and cannot be easily refined by vacuum distillation. (Tertiary amino-ester (44) was obtained by formation and removal of the N-nitroso derivative of contaminant secondary amino-ester.)

Elpern (1959) has reported the synthesis of various amino-di-esters of general structure (40) where R^1 was a phenylbutyl, phenylpropyl, or phenethyl group.

Beckett _et al_ (1959) have synthesized a series of amino-di-esters of general structure (40) for use as intermediates in the synthesis of 4-piperidones.

An interesting discussion of the difficulties in the preparation and distillation of amino-di-esters has been published by Harper _et al_ (1960). (This information is presented in the chapter entitled 'Chemistry Discussion'.)

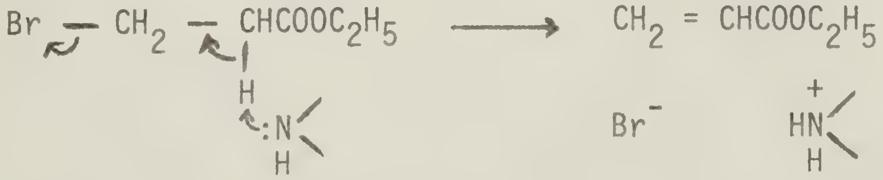
An alternate synthesis of amino-esters of general structure (38) involves the use of halo-esters and amines.

$$2\ R^1R^2NH + XCHR^3CHR^4COOR^5 \longrightarrow \underset{(38)}{R^1R^2NCHR^3CHR^4COOR^4} + R^1R^2\overset{+}{N}H_2\ X^-$$

Thayer and McElvain (1928) have synthesized ethyl

3-anilinopropionate from aniline and ethyl 3-bromopropionate using this method. One mol of ester was reacted with 2 mols of aniline at 110 - 120^0 for 2 to 3 hours giving a 75% yield of product.

Drake and McElvain (1933) have shown that the rate of reaction of alkyl bromides with piperidine is in the order primary> secondary>tertiary and that primary bromides, in general, react with 2 mols of piperidine to give one mol of tertiary amine and one mol of piperidine hydrobromide. The same investigators (1934) then extended their studies to include various bromo-esters. They ascribed the unusual reactivity of 3-bromo-esters towards piperidine to the enhanced activity of the α-hydrogen atom caused by the adjacent carbethoxy group's electromeric effect and inductive effect. On this basis, they proposed that the first step in the elimination reaction would be the removal of a proton from the α-carbon atom by the unshared electrons of the piperidine nitrogen, followed by the release of a bromide ion from the molecule. The dehydrohalogenation of ethyl 3-bromopropionate was represented in the following manner:



Weisel *et al* (1945) found during an investigation of propionic esters that the yield of ethyl 3-(*m*-chloroanilino)propionate is considerably better when the 3-bromo-ester is used than when ethyl

acrylate is employed (80% yield as compared to 29% yield).

CHAPTER 2

EXPERIMENTAL CHEMISTRY

NOTES ABOUT THE EXPERIMENTAL DATA

A Thomas Hoover capillary melting point apparatus was used to determine melting points. All melting points and boiling points are uncorrected.

Elemental analyses were performed by the analytical laboratory of the Faculty of Pharmacy and Pharmaceutical Sciences.

Infrared spectra were run on a Beckman Model 10 Infrared Spectrophotometer.

Nmr spectra were run on a Varian Model A-60D 60 MHz spectrometer. TMS was used as the internal reference in all cases. Spectra were run at operating temperature employing a sample concentration of 100 mg/0.5 ml. Carbon tetrachloride and DMSO-d_6 were used as solvents for the amino-esters and aminohydroxamic acid hydrochlorides, respectively, Chemical shifts are quoted in δ units. No attempt has been made to calculate the theoretical resonance positions of protons in complex second order spin systems. The quoted multiplet shift values indicate the centre position of the multiplet. A chemical shift range is quoted for complex, unsymmetrical multiplets.

All commercially supplied reagents were used without further purification.

1) SYNTHESIS OF AMINO-ESTERS

General Preparative Methods

Method 1 - Synthesis of Secondary Amino-esters from Bases with Moderate to High Nucleophilicity

Methyl methacrylate, methyl acrylate or methyl crotonate (0.25 mol) was added to a solution of an equimolecular quantity of the appropriate amine in anhydrous methanol (50 - 100 ml). The reaction mixture was heated under reflux with stirring until absence or diminution of C = C stretching vibrations on the reaction mixture ir (*ca*. 1630 - 1640 cm^{-1}) indicated a satisfactory degree of reaction completion. The solvent was removed *in vacuo*, and the residual oil fractionally distilled under vacuum.

Method 2 - Synthesis of Secondary Amino-esters from Bases of Relatively Low Nucleophilicity

The appropriate 3-bromo-ester (0.25 mol) was dissolved in anhydrous benzene (50 - 100 ml). To this solution, the appropriate amine (0.50 mol) was added dropwise with stirring. The reaction mixture was heated under reflux with stirring and the accumulated precipitate of amine hydrobromide was removed from time to time. When precipitation was complete, solvent was removed *in vacuo*, and the residual oil fractionally distilled under vacuum.

Method 3 - Synthesis of Tertiary Amino-esters from Bases of Relatively Low Nucleophilicity

Methyl acrylate (0.50 mol) was added to a solution of the appropriate amine (0.25 mol) in anhydrous methanol (50 - 100 ml). The solution was heated under reflux with stirring until the ir of the reaction mixture exhibited a lack of N-H stretching (*ca*. 3310 - 3500 cm^{-1}) due to secondary amine. The solvent was removed *in vacuo*. The residual oil was not distilled.

Method 4 - Synthesis of Tertiary Amino-esters from Bases of Moderately High Nucleophilicity

Methyl acrylate, methyl crotonate, or methyl methacrylate (0.25 mol) was added to a solution of amine (0.25 mol) in anhydrous methanol (50 - 100 ml). The stirred solution was cooled in ice, and then allowed to come to room temperature. Stirring was continued as the solution was brought under reflux. The reaction was judged complete when the ir of the reaction mixture exhibited a lack of N-H stretching (*ca*. 3310 - 3500 cm^{-1}). The excess solvent was removed *in vacuo*, and the crude oil was fractionally distilled under reduced pressure.

Method 5 - Synthesis of Amino-di-esters from Bases of Moderately High Nucleophilicity

A. Methyl acrylate in appreciable excess (at least 0.50 mol) was added dropwise to a stirred solution of the amine (0.25 mol) in anhydrous methanol (50 - 100 ml) cooled in an ice bath. The reaction mixture was allowed to come to room temperature, and then brought under reflux, stirring continuously. At reaction completion, as evidenced by a lack of N-H stretching (*ca.* 3310 - 3500 cm^{-1}) on the reaction mixture ir, excess solvent and acrylate were removed *in vacuo*. The residual oil was not distilled.

B. Methyl acrylate in appreciable excess (at least 0.25 mol) was added dropwise to a continuously stirred solution of the appropriate secondary amino-ester (0.25 mol) dissolved in anhydrous methanol (50 - 100 ml). At completion of the reaction, as noted by the lack of N-H stretching (*ca.* 3310 - 3500 cm^{-1}) on the reaction mixture ir, the excess solvent and acrylate were removed *in vacuo*. The residual oil was not distilled.

2) PREPARATION OF SALTS OF THE AMINO-ESTERS

The hydrochlorides and hydrobromides of the amino-esters were obtained by gassing a solution of the appropriate amino-ester in anhydrous diethyl ether with HCl or HBr dried by passage through concentrated sulfuric acid.

The precipitated salts were recrystallized from anhydrous methanol-ether, unless otherwise noted.

In some cases, both the hydrochloride and hydrobromide salts were oils, and it was necessary to prepare the acid oxalate for characterization purposes. The appropriate amino-ester was dissolved in methanol containing an exactly equivalent quantity of oxalic acid. The solution was chilled, and the resulting precipitate collected and recrystallized from methanol.

All salts were characterized by their infrared spectra and melting points, and were colorless compounds.

Methyl 3-benzylaminopropionate

Methyl acrylate (25.4 g) was added to a solution of benzylamine (31.5 g) in 50 ml anhydrous methanol. The reaction mixture was heated under reflux with stirring for 19 h. Methanol was removed *in vacuo*, and the residual oil was distilled under reduced pressure giving 43.5 g product (77% yield). The title compound had bp 123^{o} (2 mm). Reported (Southwick and Crouch, 1953) bp 145 - 147^{o} (7 mm).

Ir spectrum (film): 3350 (N-H), 1740 (C = 0) cm^{-1}.

Nmr spectrum (CCl_4): δ1.48 (s, 1, NH), 2.58 (m, 4, CH_2CH_2), 3.60 (s, 3, OCH_3), 3.70 (s, 2, $C_6H_5\underline{CH_2}$), 7.20 (s, 5, C_6H_5).

The *hydrobromide* of the title compound was recrystallized from acetone-ether and had mp 135 - 137^{o}.

Analysis - Found: C, 48.02, H, 5.77, N, 5.23.

$C_{11}H_{16}BrNO_2$ requires: C, 48.19, H, 5.88, N, 5.11.

Methyl 2-methyl-3-benzylaminopropionate

Using general method 1 for amino-ester synthesis, the title compound was obtained in 64% yield from methyl methacrylate and benzylamine. The reaction mixture was refluxed with stirring for 2 days. The product had bp 134 - 135^o (5 mm). Reported (Ganellin and Spickett, 1965) bp 96 - 98^o (0.3 mm).

Ir spectrum (film): 3320 (N-H), 1725 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.12 (d, 3, J = 7 Hz, $CHC\underline{H}_3$), 1.70 (s, 1, NH), 2.30 - 2.85 (m, 3, CH_2CH), 3.60 (s, 3, OCH_3), 3.70 (s, 1, $C_6H_5C\underline{H}_2$), 7.20 (s, 5, C_6H_5).

Analysis - Found: C, 69.77, H, 8.34, N, 6.50. $C_{12}H_{17}NO_2$ requires: C, 69.53, H, 8.27, N, 6.75.

The hydrobromide of the title compound melted at 91 - 92^o.

Methyl 3-methyl-3-benzylaminopropionate

General method 1 for amino-ester synthesis gave the above compound in 59% yield from methyl crotonate and benzylamine when the reaction mixture was heated under gentle reflux with stirring for 3 days. The product had bp 115 - 117^o (1.0 mm). Reported (McErlane, 1971) bp 120 - 123^o (0.6 mm).

Ir spectrum (film): 3350 (N-H), 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.06 (d, 3, J = 6.5 Hz, $CHC\underline{H}_3$), 1.40 (s, 1, NH, absent after D_2O exchange), 2.30 (d, 2, J = 6.5 Hz,

$CHC\underline{H}_2COOCH_3$), 3.05 (sextet, 1, J = 6 Hz, CH), 3.54 (s, 1, OCH_3), 3.68 (s, 2, $C_6H_5C\underline{H}_2$), 7.18 (s, 5, $C_6\underline{H}_5$).

Analysis - Found: C, 69.78, H, 8.02, N, 6.40. $C_{12}H_{17}NO_2$ requires: C, 69.53, H, 8.27, N, 6.75.

The hydrobromide of the title compound was recrystallized from acetone and had mp 136 - 137°.

Methyl 3-phenethylaminopropionate

Employing general method 1 for amino-ester synthesis, the title compound was obtained in 64% yield from phenethylamine and methyl acrylate. The stirred reaction mixture was not heated, but initially cooled in an ice bath, and then allowed to come to room temperature and react for a further 46 h. The product had bp 140 - 142° (3.2 mm).

Ir spectrum (film): 3350 (N-H), 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.08 (1, s, NH, absent after D_2O exchange), 2.15 - 3.10 (8, m, $C\underline{H}_2C\underline{H}_2NHC\underline{H}_2C\underline{H}_2$), 3.57 (3, s, OCH_3), 7.18 (5, s, C_6H_5).

Analysis - Found: C, 69.58, H, 8.20, N, 6.89. $C_{12}H_{17}NO_2$ requires: C, 69.50, H, 8.27, N, 6.75.

The hydrobromide of the title compound was recrystallized from acetone-methanol-ether and had mp 140 -141°.

Methyl 2-methyl-3-phenethylaminopropionate

Methyl methacrylate and phenethylamine were condensed using general method 1 for amino-ester synthesis, giving a product which distilled at 124° (1 mm). Reported (Horii *et al*, 1962) bp 136° (5 mm).

Ir spectrum (film): 3400 (N-H), 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.06 (3, d, $CHC\underline{H}_3$, seen clearly after D_2O exchange), 1.09 (1, s, NH, absent after D_2O exchange), 2.30 - 2.90 (7, m, $C\underline{H}_2C\underline{H}$ and $C_6H_5C\underline{H}_2C\underline{H}_2$), 3.52 (3, s, OCH_3), 7.10 (5, s, C_6H_5).

The hydrobromide of the above compound had mp 154 - 155° (acetone-ether).

Analysis - Found: C, 51.58, H, 6.89, N, 4.92. $C_{13}H_{20}BrNO_2$ requires: C, 51.66, H, 6.67, N, 4.63.

Methyl 3-methyl-3-phenethylaminopropionate

General method 1 for amino-ester synthesis gave the title compound in 64% yield from methyl crotonate and phenethylamine. The stirred reaction mixture was heated under reflux for 3 days. The product had bp 130 - 132° (2 mm).

Ir spectrum (film): 3340 (N-H), 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.01 (1, s, NH, absent after D_2O exchange), 1.03 (3, d, $CHC\underline{H}_3$, seen clearly after D_2O exchange),

2.26 (2, doublet of doublets, J = 1.5 and 6 Hz, $CH C\underline{H}_2 C = O$), 2.75 (1, 5, J = 6 Hz, CH), 3.54 (3, s, OCH_3), 7.14 (5, s, C_6H_5).

Analysis - Found: C, 70.85, H, 8.74, N, 6.10. $C_{12}H_{19}NO_2$ requires: C, 70.55, H, 8.65, N, 6.33.

The *hydrobromide* of the title compound had mp 146 - 147°.

Methyl 2-methyl-3-phenylpropylaminopropionate

The first general method for amino-ester synthesis gave the above compound in 61% yield from methyl methacrylate and 3-phenylpropylamine. The reaction mixture was initially chilled in an ice bath, and after coming to room temperature, was allowed to reflux with stirring for 24 h. The amino-ester had bp 153 - 155° (3 mm). Reported (Horii *et al*, 1962) bp 126 - 128° (3 mm).

Ir spectrum (film): 3350 (N-H), 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ0.91 (1, s, NH, absent after D_2O exchange), 1.10 (3, d, J = 6.5 Hz, $CHC\underline{H}_3$), 1.77 (2, q, $NHC\underline{H}_2CH(CH_3)$), 2.30 - 2.86 (7, m, $C_6H_5C\underline{H}_2C\underline{H}_2C\underline{H}_2$ and $C\underline{H}(CH_3)$), 3.58 (3, s, OCH_3), 7.11 (5, s, C_6H_5).

Analysis - Found: C, 71.30, H, 8.98, N, 5.96. $C_{14}H_{21}NO_2$ requires: C, 71.45, H, 8.99, N, 5.94.

The *hydrochloride* of the title compound had mp 114 - 115°.

Methyl 3-anilinopropionate

Using general method 2 for the synthesis of amino-esters, the title compound was prepared in 28% yield. Methyl 3-bromopropionate (57.4 g) and aniline (64.0 g) were dissolved in anhydrous benzene (50 ml), and the solution refluxed with stirring for 1 day, occasionally removing the accumulated precipitated aniline hydrobromide. After removal of benzene *in vacuo*, the crude oil was fractionally distilled under vacuum to give the title compound (17.5 g), bp 144 - 146^{0} (2.8 mm). Reported (Howton, 1945) bp 125 - 126^{0} (3 mm).

Ir spectrum (film): 3420 (N-H), 1735 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ 2.42 (2, t, J = 6.5 Hz, $C_6H_5NHC\underline{H}_2$), 3.31 (2, t, J = 6.5 Hz, $C_6H_5NHC\underline{H}_2C\underline{H}_2$), 3.56 (3, s, OCH_3), 3.88 (1, s, NH, absent after D_2O exchange), 6.20 - 7.25 (5, m, C_6H_5).

Analysis - Found: C, 66.77, H, 7.33, N, 7.81. $C_9H_{13}NO_2$ requires: C, 67.01, H, 7.31, N, 7.81.

The *hydrobromide* of the title compound was recrystallized from acetone-ether and had mp 105 - 106^{0}.

Methyl 2-methyl-3-anilinopropionate

The title compound was synthesized in 19% yield from methyl 3-bromo-2-methylpropionate and aniline, using general method 2 for amino-ester synthesis. The product had bp 124 - 126^{0} (2 mm). Reported (Bieber, 1950) bp 152^{0} (15 mm).

Ir spectrum (film): 3420 (N-H), 1730 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.18 (3, d, J = 7 Hz, $CH\underline{H_3}$), 2.76 (1, q, $\underline{CH}(CH_3)$), 3.13 (2, doublet of doublets, $NHC\underline{H_2}$), 3.63 (3, s, OCH_3), 4.78 (1, s, NH, absent after D_2O exchange), 6.80 (5, m, C_6H_5).

Analysis - Found: C, 68.19, H, 7.74, N, 7.18. $C_{11}H_{15}NO_2$ requires: C, 68.36, H, 7.82, N, 7.24.

The hydrochloride and hydrobromide of the above compound were oils. The acid oxalate had mp 121°. Reported (Bieber, 1950) mp 120°.

Methyl 3-methyl-3-anilinopropionate

The title compound was prepared from methyl 3-bromobutyrate in 28% yield using general method 2 for amino-ester synthesis. The product had bp 125 - 127° (2 mm).

Ir spectrum (film): 3390 (N-H), 1735 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.17 (3, d, J = 6.5 Hz, $CHC\underline{H_3}$), 2.42 (2, t, J = 6.5, $CHC\underline{H_2}$), 3.55 (3, s, OCH_3), 3.63 (1, s, NH, absent after D_2O exchange), 3.83 (1, q, J = 6.5 Hz, $C\underline{H}CH_3$, seen clearly after D_2O exchange), 6.68 (5, m, C_6H_5).

Analysis - Found: C, 68.59, H, 7.91, N, 7.01. $C_{11}H_{15}NO_2$ requires: C, 68.36, H, 7.82, N, 7.24.

The hydrobromide of the above compound had mp 139 - 140°.

Methyl 3-(N-methylbenzylamino)propionate

Using general method 4 for the synthesis of amino-esters, the title compound was prepared in 86% yield from N-methylbenzylamine and methyl acrylate. The stirred solution was allowed to reach room temperature, and then refluxed for 15 h. Methanol was removed *in vacuo*, and the crude oil fractionally distilled (bp 102 - 104° (1 mm)).

Ir spectrum (film): 3320 (N-H), 1735 (C = 0) cm^{-1}.

Nmr Spectrum (CCl_4): δ 2.12 (3, s, NCH_3), 2.52 (4, m, CH_2CH_2), 3.43 (2, s, $C_6H_5C\underline{H}_2$), 3.57 (3, s, OCH_3), 7.18 (5, s, C_6H_5).

Analysis - Found: C, 69.51, H, 8.26, N, 6.75. $C_{12}H_{17}NO_2$ requires: C, 69.53, H, 8.26, N, 6.75.

The *hydrochloride* of the title compound had mp 99 - 100°.

Methyl 2-methyl-3-(N-methylbenzylamino)propionate

General method 4 for amino-ester synthesis gave the title compound in 53% yield from N-methylbenzylamine and methyl methacrylate. The product had bp 100 - 102° (1 mm). Reported (Barron *et al*, 1965) bp 94° (0.3 mm).

Ir spectrum (film): 1745 (C = 0) cm^{-1}.

Nmr spectrum (CCl_4): δ 1.08 (3, d, J = 6.5 Hz, $CHC\underline{H}_3$), 2.12 (3, s, NCH_3), 2.15 - 2.85 (3, m, NCH_2CH), 3.42 (2, s, $C_6H_5C\underline{H}_2$), 3.56 (3, s, OCH_3), 7.13 (5, s, C_6H_5).

Analysis - Found: C, 70.54, H, 8.65, N, 6.35. $C_{13}H_{19}NO_2$ requires: C, 70.55, H, 8.65, N, 6.33.

The hydrochloride of the title compound had mp 149 - 150°.

Methyl 3-methyl-3-(N-methylbenzylamino)propionate

The title compound was obtained in 68% yield from N-methylbenzylamine and methyl crotonate using general method 4 for amino-ester preparation, had bp 111 - 112° (1.5 mm).

Ir spectrum (film): 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.02 (3, d, J = 7 Hz, $CHC\underline{H}_3$), 2.05 (3, s, NCH_3), 2.32 (2, doublet of quartets, J = 7 and 12 Hz, $CHC\underline{H}_2$), 3.45 (1, o, $C\underline{H}(CH_3)CH_2$), 3.54 (3, s, OCH_3), 7.15 (5, s, C_6H_5).

Analysis - Found: C, 70.55, H, 8.64, N, 6.21. $C_{13}H_{19}NO_2$ requires: C, 70.55, H, 8.65, N, 6.33.

Both the hydrochloride and hydrobromide of the title compound were oils. The acid oxalate had mp 114 - 115°.

Methyl 3-(di-2-phenylethylamino)propionate

The title compound was obtained in 95% yield from methyl acrylate and di-2-phenylethylamine using general method 3 for amino-ester synthesis. The product was not distilled, but was of analytical quality.

Ir spectrum (film): 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ2.10 - 3.10 (12, m, $(CH_2CH_2)_3N$), 3.56 (3, s, OCH_3), 7.14 (10, s, $2C_6H_5$).

The hydrobromide of the title compound was recrystallized from acetone-ether and had mp 122 - 123°.

Analysis - Found: C, 61.46, H, 6.59, N, 3.31. $C_{20}H_{26}BrNO_2$ requires: C, 61.22, H, 6.68, N, 3.57.

Methyl 3-(N-benzylphenethylamino)propionate

General method 3 for amino-ester preparation gave the title compound in 94% yield from methyl acrylate and N-benzylphenethylamine. The reaction mixture was refluxed with stirring for 36 h.

Ir spectrum (film): 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ2.55 (4, m, NCH_2CH_2), 3.50 (7, s, $OC\underline{H}_3$ and $(C_6H_5C\underline{H}_2)_2N$, accidentally equivalent), 7.22 (10, s, $2C_6H_5$).

Analysis - Found: C, 67.59, H, 6.62, N, 4.38. $C_{19}H_{24}ClNO_2$ requires: C, 67.58, H, 6.62, N, 4.49.

Methyl 3-diphenylmethylaminopropionate

General method 1 for amino-ester synthesis gave the title compound in 98% yield from diphenylmethylamine and methyl acrylate. The reaction mixture was refluxed with stirring for 45 h, and the product was not distilled. The hydrobromide of the title compound had mp 237 - 238°.

Ir spectrum (Nujol Mull): 2520 - 3200 (broad band, $\overset{+}{N}H_2$), 1735 (C = O) cm^{-1} .

Nmr spectrum (CCl_4): δ1.82 (1, s, NH, absent after D_2O exchange), 2.58 (4, m, CH_2CH_2), 3.55 (3, s, OCH_3), 4.74 (1, s, $(C_6H_5)_2C\underline{H}N$), 7.23 (10, m, $2C_6H_5$).

Analysis - Found: C, 58.37, H, 5.59, N, 3.88. $C_{17}H_{20}BrNO_2$ requires: C, 58.29, H, 5.76, N, 3.99.

Methyl 3-(1,2-diphenylethylamino)propionate

The above compound was synthesized in 96% yield from 1,2-diphenylethylamine and methyl acrylate by general method 1 for amino-ester synthesis employing a reaction time of 53 h. The product was not distilled, but was characterized _via_ the _hydrobromide_ which had mp 170 - 171°.

Ir spectrum (Nujol Mull): 2480 - 3190 (broad band, $\overset{+}{N}H_2$), 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.74 (1, s, NH, absent after D_2O exchange), 2.47 (4, m, CH_2CH_2), 2.85 (2, d, J = 7 Hz, $C_6H_5C\underline{H_2}$), 3.51 (3, s, OCH_3), 3.83 (1, t, J = 7 Hz, CH), 6.92 - 7.32 (10, m, $2C_6H_5$).

Analysis - Found: C, 59.11, H, 5.81, N, 3.82. $C_{18}H_{22}BrNO_2$ requires: C, 59.34, H, 6.09, N, 3.84.

Methyl 3-(3,3-diphenylpropylamino)propionate

The title compound was prepared in 96% yield from 3,3-diphenylpropylamine and methyl acrylate, using general method 1 for amino-ester synthesis, and a reaction time of 24 h. The product was not distilled but was of analytical purity. The hydrobromide of the title compound had mp 131 - 132°.

Ir spectrum (film): 3530 (N-H), 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.24 (1, s, NH, absent after D_2O exchange), 1.82 - 2.90 (8, m, $C\underline{H}_2C\underline{H}_2NHC\underline{H}_2C\underline{H}_2$), 3.57 (3, s, OCH_3), 4.00 (1, t, $(C_6H_5)_2C\underline{H}$), 7.15 (10, s, C_6H_5).

Analysis - Found: C, 76.69, H, 7.80, N, 4.67. $C_{19}H_{23}NO_2$ requires: C, 76.72, H, 7.79, N, 4.70.

N,N-Di-(2-carbomethoxyethyl)benzylamine

Using method 5A for amino-di-ester synthesis, the title compound was prepared in 97% yield. A large excess of methyl acrylate (100 g) was added dropwise with stirring to a solution of benzylamine (32.7 g) in dry methanol (125 ml) chilled in an ice bath. After coming to room temperature, the solution was refluxed with stirring for 4 days until there was no N-H stretching (*ca*. 3310 - 3500 cm^{-1}) visible on the ir of the reaction mixture. Excess acrylate and methanol were removed *in vacuo* giving an oil which was not distilled. Reported (Rajsner *et al*, 1963) 150 - 161° (0.6 mm).

Ir spectrum (film): 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ2.56 (8, m, $CH_2CH_2NCH_2CH_2$), 3.57 (8, s, CH_2, and $2OCH_3$, accidentally equivalent), 7.22 (5, s, C_6H_5).

Analysis - Found: C, 64.79, H, 7.40, N, 4.95. $C_{15}H_{21}NO_4$ requires: C, 64.49, H, 7.57, N, 5.01.

The hydrochloride of the title compound had mp 84 - 85°.

N,N-Di-(2-carbomethoxyethyl)phenethylamine

The title compound was obtained in 97% yield from phenethylamine and methyl acrylate using method 5A for amino-di-ester synthesis. The product was not distilled. Reported (Elpern, 1959) bp 148° (0.1 mm).

Ir spectrum (film): 1740 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ2.15 - 2.98 (12, m, $C_6H_5\underline{CH_2}\underline{CH_2}N(\underline{CH_2}\underline{CH_2}COOCH_3)_2$), 3.60 (6,s, $2OCH_3$), 7.10 (10, s, C_6H_5).

Analysis - Found: C, 65.39, H, 8.20, N, 4.83. $C_{16}H_{23}NO_4$ requires: C, 65.50, H, 7.90, N, 4.83.

The hydrochloride of the title compound had mp 99 - 100°.

N-(2-carbomethoxyethyl)-N-(2-carbomethoxypropyl)benzylamine

General method 5B for amino-di-ester synthesis gave the above compound in 91% yield. Methyl acrylate (25 g) was added dropwise to a stirred solution of methyl 2-methyl-3-benzylaminopro-

pionate (26.5 g) chilled in an ice bath. After 1 h, the mixture was brought under reflux, and after 48 h, the excess acrylate and methanol were removed *in vacuo*. The resulting oil was not distilled, but was of analytical purity. Reported (Carabatias, 1962) bp 140 - 145° (0.3 mm).

Ir spectrum (film): 1735 (C = O) cm^{-1}.

Nmr spectrum (CCl_4): δ1.05 (3, d, J = 6 Hz, $CHC\underline{H}_3$), 2.15 - 3.05 (8, m, $CH_2CH_2NCH_2CH_2$), 3.58 (8, m, $C_6H_5C\underline{H}_2$ and $2OCH_3$), 7.22 (5, s, C_6H_5).

Analysis - Found: C, 65.35, H, 7.99, N, 4.74. $C_{16}H_{23}NO_4$ requires C, 65.48, H, 7.90, N, 4.77.

The *hydrochloride* of the title compound had mp 76 - 77°.

3) SYNTHESIS OF AMINOHYDROXAMIC ACID HYDROCHLORIDES

General Preparative Methods

Method 1 - (Coutts *et al*, 1969; 1971)

A solution of the appropriate amino-ester (0.02 mol in 20 ml dry methanol) was added dropwise to a constantly stirred solution of hydroxylamine hydrochloride (0.02 mol) in anhydrous methanol (50 ml) cooled to 0°. Stirring was continued at room temperature for several hours and then at 50°. Methanol was removed *in vacuo*, and the resulting oil dried *in vacuo* over Drierite.

The dried oil was dissolved in absolute methanol and anhydrous ether added until the solution became turbid. The solution was refrigerated and the resulting precipitate collected. Any amino-ester hydrochloride present was removed by either:

A) fraction recrystallization from anhydrous methanol-ether, or

B) an acid-base extraction - the crude hydroxamate was dissolved in a few ml of distilled water, and dilute sodium hydroxide solution (10%) added until the solution was basic (pH approximately 8). The basified aqueous solution was extracted with ether to remove any amino-ester present, and the aqueous layer acidified with dilute hydrochloric acid (10%), and then concentrated *in vacuo*. Precipitated sodium chloride was removed by filtration, and the solution further concentrated to yield a crude hydroxamic acid hydrochloride. This was recrystallized several times from absolute methanol to remove any sodium chloride still present, and finally recrystallized from absolute methanol-ether.

Method 2 - (Coutts *et al*, 1969; 1971)

A cold methanolic solution of potassium hydroxide (0.12

mol in 40 ml anhydrous methanol) was added to a constantly stirred solution of hydroxylamine hydrochloride (0.18 mol in 50 ml anhydrous methanol) cooled in an ice bath. After 3 minutes the precipitated potassium chloride was removed by filtration. To the filtrate was added a methanolic solution of the appropriate ester (0.03 mol in 20 ml dry methanol). The temperature of the stirred solution was maintained at 0^{0} to 5^{0} for one hour, and then allowed to reach room temperature. Stirring was continued until the ir of an evaporated portion of the reaction mixture lacked ester carbonyl absorption (*ca*. 1720 cm^{-1}), but instead exhibited a lower frequency hydroxamate carbonyl absorption (*ca*. 1640 - 1680 cm^{-1}). Methanol was removed *in vacuo* and the resulting oil dried in a vacuum dessicator over Drierite. The resulting semi-solid was recrystallized from absolute methanol to remove any potassium chloride present. Final recrystallization from absolute methanol-ether gave aminohydroxamic hydrochlorides of acceptable purity.

The formation of a violet chelate in alcoholic ferric chloride solution was used as a qualitative test for aminohydroxamates in the reaction mixtures.

All hydroxamates were characterized by ir spectra of Nujol Mulls or KBr discs. These spectra exhibited either one or two strong absorption bands due to carbonyl stretching within the range 1640 - 1680 cm^{-1}. If present, the lower carbonyl absorption band was generally or equal or lower intensity than the higher

carbonyl band.

All aminohydroxamic acid hydrochlorides prepared in this study were recrystallized from anhydrous methanol-ether, unless otherwise noted.

The nmr spectra of all hydroxamic acid hydrochlorides were run in DMSO-d_6. These showed a 3- or 4-proton broad band in the δ8.05 to 11.10 range due to the one or two protons of the $\overset{+}{N}H$ or $\overset{+}{N}H_2$ groups, respectively, and the two protons of the NHOH group. These protons exchanged in D_2O.

3-Benzylaminopropionohydroxamic acid hydrochloride

The title compound was prepared in 68% yield employing general method 2 for hydroxamate synthesis. KOH (13.5 g) in cold anhydrous methanol (80 ml) was added to a constantly stirred solution of hydroxylamine hydrochloride (25 g) in anhydrous methanol (100 ml) cooled in an ice bath. KCl precipitate was removed, and a solution of methyl 3-benzylaminopropionate (11.6 g) in anhydrous methanol (20 ml) was added dropwise with stirring. After reaching room temperature, the reaction mixture was stirred for a further 22 h. The title compound was recrystallized from anhydrous methanol-ether and had mp 158 - 159^o. Reported (Coutts *et al*, 1969) mp 158 - 159^o.

Ir spectrum (KBr disc): 1645, 1675 (C = O) cm^{-1}.

Analysis - Found: C, 51.80, H, 6.34, N. 12.13.

$C_{10}H_{15}ClN_2O_2$ requires; C, 52.06, H, 6.55, N, 12.14.

2-Methyl-3-benzylaminopropionohydroxamic acid hydrochloride

The above compound was synthesized in 41% yield *via* general method 2 for aminohydroxamate synthesis, using a reaction time of 36 h. The product had mp 166 - 167°.

Ir spectrum (KBr disc): 1675 (C = O) cm^{-1}.

Analysis - Found: C, 54.09, H, 6.95, N, 11.17.

$C_{11}H_{16}ClN_2O_2$ requires: C, 53.97, H, 7.00, N, 11.44.

Attempted preparation of 3-methyl-3-benzylaminopropionohydroxamic acid hydrochloride

General method 2 for hydroxamate synthesis gave an oil which could not be recrystallized or characterized *via* reineckate formation.

3-Phenethylaminopropionohydroxamic acid hydrochloride

General method 2 for aminohydroxamate preparation gave the title compound in 35% yield. The reaction mixture was stirred for 24 hours at room temperature. The title compound had mp 149 - 150°.

Ir spectrum (KBr disc): 1640, 1670 (C = O) cm^{-1}.

Analysis - Found: C, 54.05, H, 6.83, N, 11.17.

$C_{11}H_{17}ClN_2O_2$ requires: C, 53.97, H, 7.00, N, 11.44.

2-Methyl-3-phenethylaminopropionohydroxamic acid hydrochloride

The above compound was obtained in 83% yield using general method 2 for aminohydroxamate preparation with a reaction time of 36 h. The product had mp 135 - 136°. Reported (Coutts *et al*, 1971) mp 126 - 127°.

Ir spectrum (KBr disc): 1650, 1685 (C = O) cm^{-1}.

Analysis - Found: C, 55.95, H, 7.32, N, 10.88.

$C_{12}H_{19}ClN_2O_2$ requires: C, 55.70, H, 7.40, N, 10.82.

3-Methyl-3-phenethylaminopropionohydroxamic acid hydrochloride

The title compound was obtained in 31% yield using general method 2 for aminohydroxamate synthesis and had mp 131 - 132°. A reaction time of 24 h was employed.

Ir spectrum (KBr disc): 1650, 1675 (C = O) cm^{-1}.

Analysis - Found: C, 55.61, H, 7.12, N, 10.95.

$C_{12}H_{19}ClN_2O_2$ requires: C, 55.70, H, 7.40, N, 10.82.

2-Methyl-3-phenylpropylaminopropionohydroxamic acid hydrochloride

General method 2 for aminohydroxamate synthesis was

employed, giving the title compound in 47% yield after a reaction time of 24 h. The product had mp 130 - 131°.

Ir spectrum (KBr disc): 1665 (C = O) cm^{-1}.

Analysis - Found: C, 57.22, H, 7.84, N, 10.16.

$C_{12}H_{19}ClN_2O_2$ requires: C, 57.23, H, 7.76, N, 10.26.

3-(N-methylbenzylamino)propionohydroxamic acid hydrochloride

The title compound was obtained as an oil employing general method 2 for aminohydroxamate synthesis after a reaction time of 38 h. The oil was not successfully recrystallized. The reineckate salt had mp 140 - 141°.

Ir spectrum (film): 1660 (C = O) cm^{-1}.

Analysis - Found: C, 33.97, H, 4.80. $C_{15}H_{23}CrN_8O_2S_4$ requires: C, 34.13, H, 4.97.

2-Methyl-3-(N-methylbenzylamino)propionohydroxamic acid hydrochloride

General method 1 for aminohydroxamate synthesis gave the title compound in 17% yield. To a constantly stirred cold solution of hydroxylamine hydrochloride (2 g in 50 ml anhydrous methanol) was added dropwise a solution of methyl 2-methyl-3-benzylaminopropionate (6.5 g in 25 ml anhydrous methanol). Stirring was continued for 4 days at room temperature, and for an additional 3 days at 50°. Methanol was removed, and the resulting oil was dried and

then recrystallized from absolute methanol-ether to give the title compound (1.2 g) with mp 168 - 169°.

Ir spectrum (Nujol Mull): 1665 (C = O) cm^{-1}.

Analysis - Found: C, 55.43, H, 7.35, N, 10.81.

$C_{12}H_{19}ClN_2O_2$ requires: C, 55.70, H, 7.40, N, 10.83.

Attempted preparation of 3-methyl-3-(N-methylbenzylamino)propionohydroxamic acid hydrochloride

General method 2 for aminohydroxamate synthesis gave an oil which could not be recrystallized. Attempts to characterize the oil *via* the reineckate salts were unsuccessful.

3-(di-2-phenylethylamino)propionohydroxamic acid hydrochloride

The product was obtained in 4% yield using general method 1 for aminohydroxamate synthesis. Extensive fractional recrystallization from ethanol-ether was necessary to obtain the title compound having mp 131 - 132°.

Ir spectrum (KBr disc): 1665 (C = O) cm^{-1}.

Analysis - Found: C, 65.46, H, 7.03, N, 8.16.

$C_{19}H_{25}ClN_2O_2$ requires: C, 65.40, H, 7.22, N, 8.03.

Attempted preparation of 3-dibenzylaminopropionohydroxamic acid hydrochloride

General method 2 for aminohydroxamate synthesis gave an oil which could not be recrystallized. Attempts to characterize the oil *via* reineckate formation were unsuccessful.

3-(1,2-Diphenylethylamino)propionohydroxamic acid hydrochloride

By using general method 1 for aminohydroxamate synthesis, the title compound was obtained in 2% yield, using a reaction time of 36 h. The crude product was purified by acid-base extraction. The title compound had mp 211 - 212^{o}.

Ir spectrum (KBr disc): 1670 (C = O) cm^{-1}.

Analysis - Found: C, 63.39, H, 6.54, N, 81.89.

$C_{17}H_{21}ClN_2O_2$ requires: C, 63.64, H, 6.60 , N, 81.73.

Attempted preparation of 3-(3,3-diphenylpropylamino)propionohydroxamic acid hydrochloride

General method 2 for aminohydroxamate synthesis gave an oil which could not be recrystallized or characterized as a reineckate.

Attempted preparation of N-benzyliminodipropionohydroxamic acid hydrochloride

General method 2 for aminohydroxamate preparation gave an

oil which resisted repeated attempts at recrystallization and characterization as a reineckate.

N-phenethyliminodipropionohydroxamic acid hydrochloride

General method 2 for hydroxamate synthesis gave the title compound as a viscous oil which could not be recrystallized. The reineckate salt had mp 131 - 132°.

Ir spectrum (film): 1670 (C = O) cm^{-1}.

Analysis - Found: C, 35.16, H, 4.42, N, 20.60.

$C_{18}H_{28}CrN_9O_4S_4$ requires: C, 35.16, H, 4.59, N, 20.51.

4) MISCELLANEOUS SYNTHESES

Di-2-phenylethylamine

A solution of 2-phenylethyl bromide (19.1 g) in dry benzene (25 ml) was added dropwise to a stirred solution of phenethylamine (25.0 g) in dry benzene (50 ml). After 2 weeks reaction at room temperature, amine hydrobromide precipitate was removed by filtration, and the benzene solution heated under reflux for 2 days. Benzene was removed *in vacuo*, and the residual oil distilled under reduced pressure (bp 146 - 150° (0.3 mm)). Reported (v. Braun *et al*, 1923) bp 195° (18 mm).

Analysis - Found: C, 85.26, H, 8.46, N, 6.33.

$C_{10}H_{19}N$ requires: C, 85.28, H, 8.50, N, 6.21.

N-Benzyl-2-phenylethylamine

A synthesis similar to that used to prepare di-2-phenylethylamine gave the title compound in 43% yield from 2-phenylethylbromide and benzylamine. The title compound had bp 124^o (0.15 mm). Reported (v. Braun et al, 1923) 327 - 328^o (600 mm).

Analysis - Found: C, 85.48, H, 8.35, N, 6.67. $C_9H_{17}N$ requires: C, 85.24, H, 8.11, N, 6.63.

Methyl 3-bromo-2-methylpropionate

Methyl methacrylate (183 g), hydroquinone (2 g) and benzoyl peroxide (2 g) were dissolved in carbon tetrachloride (100 ml). HBr gas was passed through the stirred solution for 2 h. The carbon tetrachloride was removed in vacuo, and the residual liquid distilled to give 278 g product (84% yield) bp 55 - 56^o (7 mm). Reported (Price and Coyner, 1940) bp 67^o (17 mm).

Methyl 3-bromopropionate

The method of Price and Coyner (1940) gave the title compound in 88% yield from methyl acrylate. The title compound had bp 78 - 79^o (26 mm). Reported (Moureu et al, 1921) bp 80^o (27 mm).

Methyl 3-bromobutyrate

The method of Price and Coyner (1940) gave the title compound in 76% yield using methyl crotonate. The product had bp 78 - 79° (22 mm). Reported (Nomura *et al*, 1968) bp 70 - 74° (18 mm).

CHAPTER 3

CHEMISTRY DISCUSSION

1) SYNTHESIS OF METHYL ARYL- AND ARYLALKYL-AMINOPROPIONATES

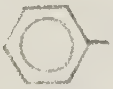
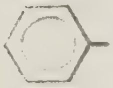
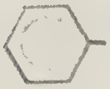
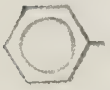
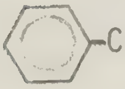
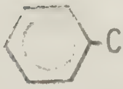
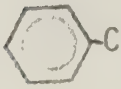
Various acylclic hydroxamic acids may be prepared from the reaction of the appropriate ester and hydroxylamine.

The following amino-esters of general formula (38) (where in all cases R^5 is a methyl group) were prepared for use in hydroxamate synthesis:

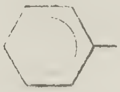
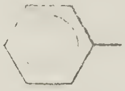
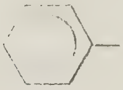
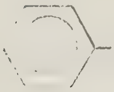
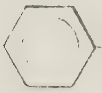
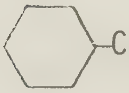
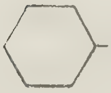
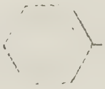
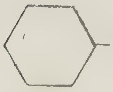
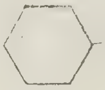
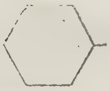
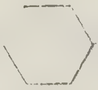
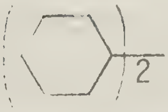
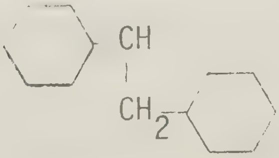
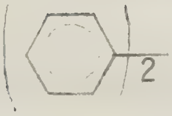
Methyl aryl- and arylalkyl-aminopropionates

$$R^1R^2NCHR^3CHR^4COOR^5$$

(38)

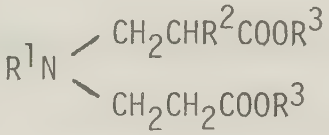
Compound	R^1	R^2	R^3	R^4
45	 CH_2	H	H	H
46	 CH_2	H	H	CH_3
47	 CH_2	H	CH_3	H
48	 CH_2CH_2	H	H	H
49	 CH_2CH_2	H	H	CH_3
50	 CH_2CH_2	H	CH_3	H
51	 $CH_2CH_2CH_2$	H	H	CH_3

Methyl aryl- and arylalkyl-aminopropionates (cont'd)

Compound	R^1	R^2	R^3	R^4
52		H	H	H
53		H	H	CH_3
54		H	CH_3	H
55	-CH_2	CH_3	H	H
56	-CH_2	CH_3	H	CH_3
57	-CH_2	CH_3	CH_3	H
58	-CH_2CH_2	-CH_2CH_2	H	H
59	-CH_2CH_2	-CH_2	H	H
60	-CH_2	-CH_2	H	H
61	 CH	H	H	H
62		H	H	H
63	 CH CH_2CH_2	H	H	H

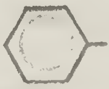
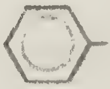
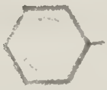
Several amino-di-esters of general formula (40) were also synthesized for use in hydroxamate synthesis:

Amino-di-esters



$$R^1N\begin{cases} CH_2CHR^2COOR^3 \\ CH_2CH_2COOR^3 \end{cases}$$

(40)

Compound	R^1	R^2	R^3
64	 $-CH_2$	H	CH_3
65	 $-CH_2CH_2$	H	CH_3
66	 $-CH_2$	CH_3	CH_3

Two general methods were employed in the present study to prepare the amino-esters listed above:

(i) by the addition of amines to methyl α,β-unsaturated esters (Adamson, 1950), i.e.,

$$R^1R^2NH + CHR^3 = CHR^4COOCH_3 \longrightarrow R^1R^2NHCHR^3CHR^4COOCH_3$$

(ii) through the reaction of a halo-ester with an amine

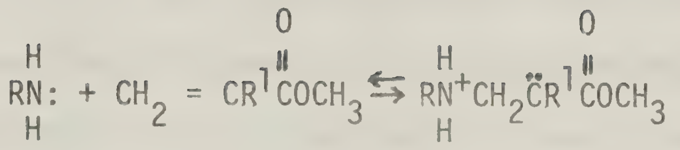
(Drake and McElvain, 1934; Weisel _et al_, 1961; Howton, 1945; Cruickshank _et al_, 1961), i.e.,

$$R^1R^2NH + XCHR^3CHR^4COOCH_3 \longrightarrow R^1R^2NHCHR^3CHR^4COOCH_3 + HX$$

Because of the simplicity of the first method, it was employed in the preparation of most of the amino-esters required in this study.

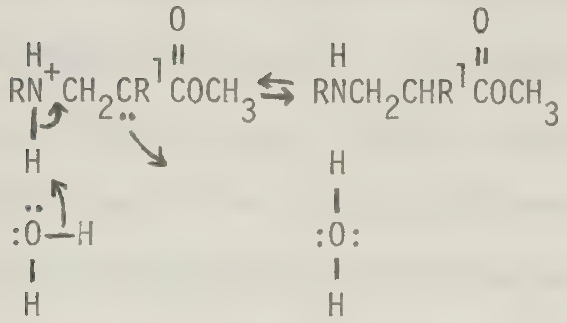
It has been noted (Bieber, 1954) that the ease of addition of amines to methyl methacrylate is in the order: piperidine> diethylamine>aniline>N-ethylaniline, and that the basicity of the amine, as well as steric hindrance, are crucial factors in the addition process. The fact that aniline failed to give an addition product with methyl methacrylate, for example, illustrates that the Michael addition cannot be used exclusively for the synthesis of amino-esters, as there are sometimes factors present which preclude its use.

The fact that certain amines do not add readily to methyl methacrylate or methyl crotonate can be partially explained if it is accepted that the Michael addition is subject to catalysis similar to that proposed for the cyanoethylation of _tert_-carbinamines by Taylor _et al_ (1959). The proposed mechanism consists of an initial attack by an electron pair at the β-carbon atom, and a subsequent rate-determining proton transfer to complete the reaction.



$$R\overset{H}{\underset{H}{N}}: + CH_2 = CR^1\overset{O}{\overset{\|}{C}}OCH_3 \rightleftarrows R\overset{H}{\underset{H}{N^+}}CH_2\ddot{C}R^1\overset{O}{\overset{\|}{C}}OCH_3$$

fast



$$\begin{array}{l} R\overset{H}{N^+}CH_2\underset{\cdot\cdot}{C}R^1\overset{O}{\overset{\|}{C}}OCH_3 \\ \quad H \\ \quad \ddot{O}-H \\ \quad H \end{array} \rightleftarrows \begin{array}{l} RNCH_2CHR^1\overset{O}{\overset{\|}{C}}OCH_3 \\ \quad H \\ \quad :O: \\ \quad H \end{array}$$

slow

It has been established (Luskin et al, 1956; Hughes, 1960) that sterically hindered amines undergo addition to esters of α,β-unsaturated acids less readily than unhindered amines. Unhindered amines may themselves act as proton transfer agents in the addition process, as outlined above. A sterically hindered amine will not easily approach a similar sterically hindered intermediate. Such a sterically hindered intermediate could result from, for example, the presence of a methyl group at the α- or β- position on the ester carbon chain. Therefore, it may be that one reason for Michael addition failure is steric interference with the proton transfer catalysis.

Another factor of crucial importance in the addition of sterically hindered amines or amines of moderate nucleophilicity to α,β-unsaturated systems is α- or β- alkyl substitution on the ester. For example, in the present study reactions involving methyl acrylate

invariably afforded a greater yield of amino-ester product than did those involving methyl methacrylate or methyl crotonate. The α-methyl group of methyl methacrylate would be expected to offer greater steric hindrance in the rate-determining proton transfer step of the Michael addition. In the addition to the steric requirements of the α-methyl group of methyl methacrylate, the differences in the stabilities of the transition states of the reactions involving methyl methacrylate and methyl acrylate must be considered. The transition state of methyl acrylate involves a secondary carbanion, whereas the carbanion involved in methacrylate addition is tertiary. The positive inductive effect of the α-methyl group in methyl methacrylate would be expected to destabilize the tertiary carbanion, making addition more difficult. It has been reported (Friedman and Wall, 1966) that since tertiary carbanions are less stable than secondary carbanions, the rate of methyl methacrylate addition to amines should be expected to be lower than that of methyl acrylate on purely electronic grounds.

The same authors point out that model studies show that the steric hindrance in methyl methacrylate addition is smaller than in methyl crotonate addition, and indeed, of less importance. For methyl crotonate, the steric effect of the β-methyl group is thought to predominate. Indeed, the positive inductive effect of the β-methyl group would be expected to somewhat destabilize the intermediate tertiary carbanion, but this factor is not as important

as in the case of methacrylate addition.

The ease of addition of amines to α,β-unsaturated esters also depends greatly upon the basicity of the amine. This is obvious from an examination of the order of ease of addition of the various amines mentioned in the study by Bieber (1954). Piperidine, the strongest of the bases used, gave the best yields, whereas aniline, a weakly basic aromatic amine, gave a very small yield of product. Thus empirically at least, basicity is a guide to ease of addition of amines to α,β-unsaturated esters. On an electronic basis, the Michael addition involves an initial nucleophilic attack by an amino nitrogen bearing a lone pair of electrons onto the terminal carbon of the α,β-unsaturated chain. A delocalization of the lone pair of electrons on amino nitrogen, as is the case in aromatic amines, discourages initial nucleophilic attach by the amine, and destabilizes the carbanion intermediate, should attack occur.

In the present study several arylalkylamino-esters containing bulky phenyl substituted side-chains were synthesized. Early in the investigation, it was found that while there was no difficulty in achieving some measure of addition of various arylalkylamines and methyl methacrylate or crotonate, the reactions would not go to completion. It was also found that purification of these high boiling amino-esters was difficult, as distillation invariably resulted in decomposition of the desired product.

Dibenzylamine, for example, combined with methyl methacrylate, as evidenced by gradual diminution of N-H stretching on the infrared spectrum of the reaction mixture. Attempts to distill the reaction mixture after removal of solvent gave unclear results. Dibenzylamine and not the desired amino-ester was obtained.

Initially, it was thought that the aforementioned reaction was subject to steric hindrance due to the bulky phenyl groups of dibenzylamine and the α-methyl group of methacrylate, but it was later established that the desired amino-ester had decomposed during distillation, giving dibenzylamine and methacrylate, the starting materials. At this point, the use of methacrylate and crotonate as α,β-unsaturated esters in Michael additions with bulky arylalkylamines was discontinued. Methyl acrylate, which, because of the steric and electronic considerations mentioned previously, had a very high reactivity towards most amines (even aniline) was, therefore, used exclusively in the production of amino-esters which, because of their high boiling points, were unlikely to distill without decomposition.

Thus it was possible to obtain an amino-ester in almost 100% yield without distillation. It was apparent that this technique would work best with secondary amines, because a large excess of acrylate could be employed to give a product free of contamination from amino-di-ester. At reaction completion, judged by disappearance of N-H stretching on the reaction mixture ir spectrum, excess

acrylate was removed in vacuo to give almost a theoretical yield of product tertiary amino-ester.

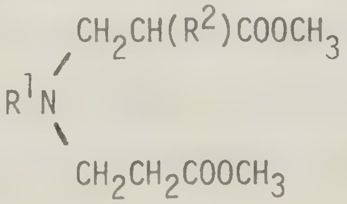
$$\underset{\text{secondary amine}}{R^1R^2NH} + \underset{\text{large excess}}{CH_2 = CHCOOCH_3} \longrightarrow \underset{\text{theoretical yield}}{R^1R^2NCH_2CH_2COOCH_3}$$

In contrast, with primary amines the quantity of acrylate used in the addition process was critical, except in those cases where distillation was possible. An excess of acrylate would give some amino-di-ester, but too little acrylate would give product amino-ester contaminated with unreacted amine. It was found in practice that with primary amines, unless distillation was possible, the resulting amino-esters were not of analytical quality (although close to it). In these cases, a hydrobromide salt was used to characterize the secondary amino-ester product.

$$\underset{\text{primary amine}}{R^1NH_2} + \underset{\text{one equivalent}}{CH_2 = CHCOOCH_3} \longrightarrow \underset{\text{impure (unless distilled)}}{R^1NHCH_2CH_2COOCH_3}$$

$$\text{plus} \quad R^1NH_2 \quad \text{or} \quad R^1N(CH_2CH_2COOCH_3)_2$$

Several amino-di-esters of general formula (40) were prepared without use of distillation.



$$R^1N\begin{cases} CH_2CH(R^2)COOCH_3 \\ CH_2CH_2COOCH_3 \end{cases}$$

(40)

The synthesis used was simply a variation of general method (1) described on page 62 . One equivalent of arylalkylamine was reacted with at least two equivalents of acrylate to generate an amino-di-ester where R^2 was H. Because of the high reactivity of acrylate, the product was obtained in almost theoretical yield and was of analytical quality.

Alternatively, to generate a branched amino-di-ester where R^2 was a methyl group, one equivalent of a previously synthesized branched chain secondary amino-ester was reacted with one equivalent of acrylate, again giving a product in almost theoretical yield and of analytical quality, without use of distillation.

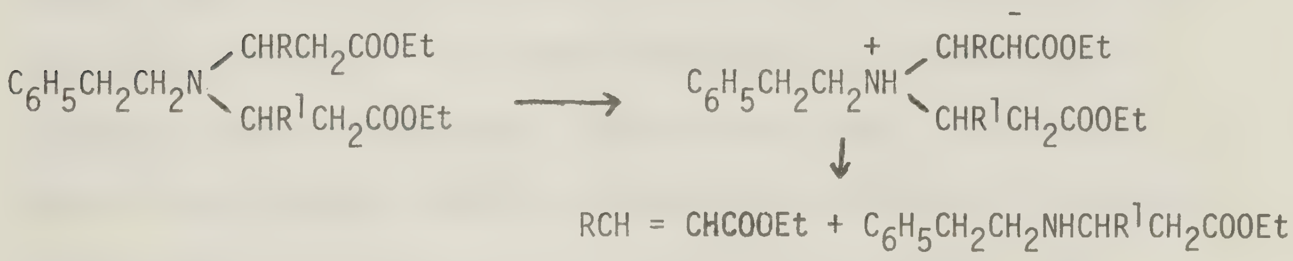
Because of the lower reactivity of methacrylate versus acrylate, it is doubtful that treatment of an unbranched aminopropionate with methyl methacrylate would give similar results (*i.e.*, a complete reaction). However, this point was not clarified during the present study.

It must be noted that several of the amino-di-esters prepared in this study have been distilled under high vacuum (Elpern, 1959; Rajsner, 1963). Distillation was avoided in the current investigation wherever possible due to the inherent product decomposition and reduction in yields.

The distillation difficulties encountered in this study have been intimated in the literature. McElvain and Stork (1946) have reported that condensations of amines with unsaturated esters

are reversible. Harper et al (1960) have reported on the decomposition of tertiary amino-di-esters during distillation. These authors state that distillation of the product obtained by the reaction of ethyl 3-methyl-2-phenylethylaminopropionate with ethyl acrylate gave ethyl crotonate as one fraction, in an amount which increased with the distillation temperature. They have suggested that the initial step in the decomposition is loss of a proton from the α-carbon of an ester moiety, the proton combining with the amino-nitrogen to give a charged complex.

$R^1 = Me$
(or vice versa)
$R = H$



$$C_6H_5CH_2CH_2N\left\langle\begin{array}{l}CHRCH_2COOEt\\CHR^1CH_2COOEt\end{array}\right. \longrightarrow C_6H_5CH_2CH_2\overset{+}{N}H\left\langle\begin{array}{l}CHR\overset{-}{C}HCOOEt\\CHR^1CH_2COOEt\end{array}\right.$$

$$\downarrow$$

$$RCH = CHCOOEt + C_6H_5CH_2CH_2NHCHR^1CH_2COOEt$$

Electron flow from the crotonate moiety is claimed to be greater, resulting in its elimination. Steric crowding by the crotonate methyl group is also said to favor this elimination.

In contrast to the room temperature conditions used in the synthesis of amino-esters from lower straight chain amines, branched amines, and alicyclic amines (Ziering et al, 1947), vigorous reflux conditions were, in general, necessary to obtain satisfactory yields of arylalkyamino-esters in the current study. This observation

could be predicted from consideration of the generally low nucleophilicity of the arylalkylamines *vis à vis* the alkylamines.

The use of methanol as a solvent in amino-ester synthesis by method (i) (described on page 62) was prompted by several considerations. First, it has been proposed by various authors (Taylor *et al*, 1959; Hughes, 1960; Montgomery and Hughes, 1961; Sanui and Ogata, 1967; Sanui *et al*, 1968) that active hydrogen compounds such as the lower alcohols, amines and water have an accelerating effect on the addition of amines to α,β-unsaturated systems. In view of reports by several workers (Vystrcil and Hudecek, 1950; Bieber, 1954; Coutts *et al*, 1971) concerning the ease of hydrolysis of many aminopropionates to their corresponding amino acids or betaines, it was deemed unadvisable to use water as a catalyst in the present work. Methanol was used, therefore, in view of its reported catalytic value, but no conclusions have been drawn about its efficacy in this regard in the present study.

Secondly, methanol was chosen as a solvent due to the desirability of preventing any ester interchange in the amino-ester product. Such ester interchange may be represented as follows:

$$R^1NHCHR^2CHR^3COOR^4 + R^5OH \longrightarrow R^1NHCHR^2CHR^3COOR^5 + R^4OH$$

Thirdly, methanol was used because of its high polarity and excellent solvation of amines and esters. The availability of large quantities of anhydrous methanol was also a factor in its choice as a solvent.

The major utility of method (ii) (described on page 63), is that it allows the preparations of various amino-esters which, for reasons already noted (see page 63) cannot be prepared in adequate yield using the Michael addition.

The use of halo-esters in the synthesis of amino-esters is demonstrated by contrasting the synthesis of anilinopropionates by the Michael addition and bromo-ester routes. Methyl 3-anilinopropionate (52) has been prepared in good yield (Howton, 1945) by the Michael addition of methyl acrylate and aniline. In contrast, the reaction of aniline, an aromatic amine of low basicity, with methyl methacrylate, has been found (Bieber, 1954) to give a low yield of methyl 2-methyl-3-anilinopropionate (53) contaminated with amides and polymers. In the course of the present investigation, methyl 2-methyl-3-anilinopropionate (53) was obtained in good yield from aniline and methyl 3-bromo-2-methylpropionate. Similarly, methyl 3-methyl-3-anilinopropionate (54) was obtained in good yield from aniline and methyl 3-bromobutyrate.

The major difficulty encountered in preparation of amino-esters from halo-esters is formation of hydrogen halide salts of both the reactant amine and the amino-ester product, thereby reducing yields and producing a mixture of products. This difficulty was overcome in this study by employing an excess (two equivalents) of amine for each equivalent of halo-ester employed. In this manner, salt formation predominates on the moderately basic nitrogen of the excess amine starting material and not on the less basic nitrogen

of the amino-ester product. The amine salt formed was easily removed by filtration, and the residual oil distilled to obtain an amino-ester of high purity. Use of excess amine in a similar fashion has been reported (Cruickshank et al, 1961) in a synthesis wherein 4 equivalents of amine were used in the preparation of δ-dialkylaminobutyrates from methyl 4-iodobutyrate.

The halo-esters used in method (ii) were easily obtained from the corresponding α,β-unsaturated esters via the method of Price and Coyner (1940). Dry hydrogen bromide was passed through a solution of methyl acrylate, methyl methacrylate, or methyl crotonate in carbon tetrachloride containing trace quantities of benzoyl peroxide and hydroquinone.

The yield of the various amino-esters prepared in the present study ranged from 19% to 98%. Reactions which employed methyl acrylate gave higher percentage yields than those using methyl methacrylate or methyl crotonate. For example, benzylamine combined with methyl acrylate in 77% yield, with methyl methacrylate in 64% yield and with methyl crotonate in 59% yield (yields after distillation). These observations are in agreement with the previously mentioned steric and electronic considerations regarding the nature of the α,β-unsaturated ester employed in the Michael addition.

Reactions using a base of moderate nucleophilicity gave higher yields than those in which weakly basic amines were used. Interaction of aniline and methyl methacrylate gave no product in

the present study, for example, while phenethylamine and methyl methacrylate combined in 64% yield.

There does not seem to be a demonstrable case of an addition inhibited by a sterically hindered amine in the present investigation.

The yields obtained in synthesis of the anilinopropionates using method (ii) were satisfactory, but an insufficient number of compounds were synthesized *via* this route to allow conclusions about the factors affecting yields obtained by this method.

The formation of amides as by-products in the synthesis of amino-esters *via* method (i) has been reported by several workers (Morsch, 1932; David *et al*, 1965; Coutts *et al*, 1971). These reports suggest that amide formation is most troublesome when high reflux temperatures and prolonged reaction times are employed. The formation of amide by-products was, indeed, noted in the present investigation, these compounds being isolated during distillation or removal of methanol *in vacuo*. The yields of amides were always small and no attempt was made to isolate or characterize the amide by-products.

All aryl- and arylakyl-aminopropionates synthesized in this study were colorless or light yellow oils with a characteristic odor. Many of these amino-esters deposited colorless crystalline solids when stored in a dessicator at room temperature. Coutts *et al* (1971) identified compounds formed in this manner as 3-amino acids

resulting from hydrolysis of amino-esters by trace amounts of water. Similar observations have been reported by Bieber (1954) who stated that 2-methyl-3-piperidinopropionate on long standing in moist air or on shaking with cold water gave the corresponding free acid. No attempt was made in the current study to isolate or identify any of the amino acids formed in this manner.

2) PREPARATION OF AMINO-ESTER SALTS

All of the amino-esters prepared were characterized as hydrochloride, hydrobromide or acid oxalate salts. It was found during the early stages of the investigation that some amino-ester hydrochlorides were appreciably hygroscopic, and difficult to recrystallize, handle and store. In most cases, therefore, the far less hygroscopic hydrobromide salt was prepared for characterization purposes, the latter being much easier to recrystallize from anhydrous methanol-ether. The hydrobromides and hydrochlorides of two amino-esters synthesized in the present study, namely, methyl 2-methyl-3-anilinopropionate (53) and methyl 2-methyl-3-(N-methylbenzylamino)propionate(46), were non-recrystallizable oils. These amino-esters were characterized by preparation of their acid oxalates.

The salts were, in general, stable and no discoloration was noted over a period of a few months. None of their melting points were accompanied by decomposition.

Most of the salts were very soluble in water, but the acid oxalates had noticeably lower water solubility.

Recrystallization of the hydrogen halide salts was generally straightforward, once the appropriate solvent system had been determined. The use of only anhydrous solvents was critical for success in these recrystallizations. Generally, the hydrogen halide salt was first obtained as an oil, despite the fact that anhydrous ether was used as a gassing medium, and that only gas dried by passage through concentrated sulfuric acid was used. Prior to final recrystallization, the oil was dried for a few days over Drierite in a vacuum dessicator, or in a gently heated flask evacuated by a vacuum pump. The dried oil was usually dissolved in a minimum quantity of anhydrous methanol, and anhydrous ether added *via* pipette until the solution became turbid. The recrystallization solution was then set aside for a few minutes, and after reaching room temperature, was refrigerated. After collection *via* filtration, the recrystallized salts were dried in a vacuum dessicator containing concentrated sulfuric acid and potassium hydroxide pellets to remove any traces of atmospheric water.

The acid oxalates were easily recrystallized from methanol.

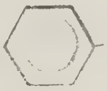
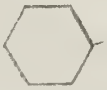
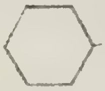
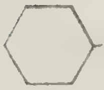
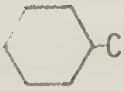
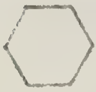
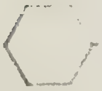
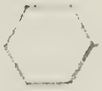
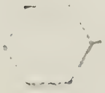
3) SYNTHESIS OF VARIOUS ARYLALKYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDES

An attempt was made to synthesize the following arylalkylaminopropionohydroxamic acid hydrochlorides of general structure (67) from the corresponding amino-esters:

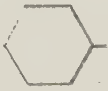
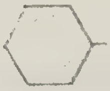
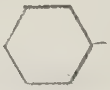
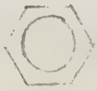
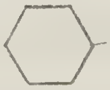
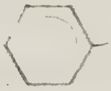
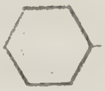
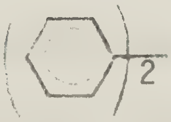
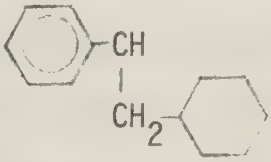
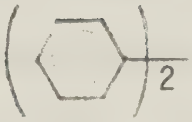
Arylalkylaminopropionohydroxamic acid hydrochlorides

$$R^1R^2\overset{+}{N}HCHR^3CHR^4CONHOH\ Cl^-$$

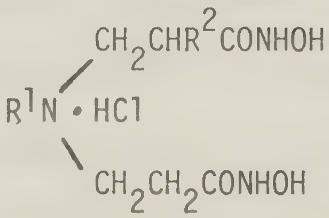
(67)

Compound	R^1	R^2	R^3	R^4
68	-CH_2	H	H	H
69	-CH_2	H	H	CH_3
70	-CH_2	H	CH_3	H
71	-CH_2CH_2	H	H	H
72	-CH_2CH_2	H	H	CH_3
73	-CH_2CH_2	H	CH_3	H
74	-$CH_2CH_2CH_2$	H	H	CH_3
75	-CH_2	CH_3	H	H
76	-CH_2	CH_3	H	CH_3

Arylalkylaminopropionohydroxamic acid hydrochlorides

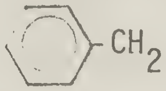
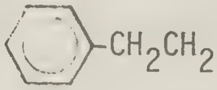
Compound	R^1	R^2	R^3	R^4
77	 -CH$_2$	CH$_3$	CH$_3$	H
78	 -CH$_2$CH$_2$	 -CH$_2$CH$_2$	H	H
79	 -CH$_2$CH$_2$	 -CH$_2$	H	H
80	 -CH$_2$	 -CH$_2$	H	H
81	 CH	H	H	H
82		H	H	H
83	 CH	H	H	H

Attempts were also made to synthesize the following aminohydroxamates of general structure (84) from the corresponding amino-di-esters:



$$R^1N\cdot HCl\begin{cases}CH_2CHR^2CONHOH\\CH_2CH_2CONHOH\end{cases}$$

(84)

Compound	R^1	R^2
85		H
86		H

The aminohydroxamic acid hydrochlorides synthesized during the present investigation were all colorless solids which did not decompose when stored in vacuo over a period of several months.

The infrared spectra of these compounds showed one or two carbonyl stretching absorption bands between 1640 and 1680 cm^{-1}. If present, the lower carbonyl band was invariably of equal or lower intensity than the higher carbonyl band. Coutts et al (1968) have explained the presence of two carbonyl absorption bands in the ir spectra of some hydroxamates on the basis of hydrogen-bonding, intra-molecularly hydrogen-bonded carbonyl groups absorbing infrared radiation at a lower frequency than the corresponding inter-molecularly bonded carbonyl group.

The nmr spectra of the aminohydroxamic acid hydrochlorides were run in DMSO-d_6. All spectra displayed in 3- or 4-proton broad signal in the δ 8.03 to 11.10 range which collapsed after addition of D_2O to the sample. This signal was attributed to the two protons of the NHOH group and the one or two protons of the $\overset{+}{N}H$ or $\overset{+}{N}H_2$ groups,

respectively. These findings are in agreement with those reported by Coutts *et al* (1971) who examined acyclic hydroxamic acids of general structure (2). These workers observed three 1-proton broad signals within the δ8.33 - 11.66 range which disappeared after addition of D_2O to the sample. The two protons of the NHOH group reportedly came to resonance in the ranges δ8.68 - 9.00 and δ10.05 - 10.58. A signal appearing at δ10.75 - 11.05 was assigned as that of the $\overset{+}{NH}$ proton, and was absent from the spectra of the corresponding bases.

Those hydroxamates which failed to recrystallize because of their extreme hygroscopicity were not easily characterized. Attempts to form reineckate salts of these hydroxamates were, in general, unsuccessful. This difficulty was undoubtedly due to presence of unreacted material and other impurities in the hydroxamate oils, giving reineckate derivatives which were analytically impure. Attempts to obtain hydroxamic acid hydrobromides by freeing the hydroxamate base and treatment with HBr were unsuccessful, again giving compounds which did not analyze correctly.

The hydroxamates of the three anilinopropionates previously prepared in this study were not synthesized, since preliminary pharmacological evaluation of the precursors showed them to be inactive as hypotensive agents.

In summary, synthetic difficulties (mainly involving product purification) prevented the synthesis of a complete series of branched and unbranched amino-esters. Similar technical problems prevented the preparation of several aminohydroxamic acids. However, despite these difficulties, a number of new compounds of interesting chemical structure were obtained for pharmacological evaluation.

CHAPTER 4

EXPERIMENTAL PHARMACOLOGY

1) BIOLOGICAL METHODS

Anaesthetized Rat Experiments

The compounds synthesized in the present study were evaluated for their effect on the blood pressure of anaesthetized rats weighing from 180 to 540 g. The animals were placed under general anaesthesia by ip injection of a 25% solution of urethane (1.88 g/kg). The surgical preparation followed procedures given by D'Amour *et al* (1965). The animal was fastened to a dissecting board by each limb using rubber bands. A mid-line incision was made along the throat from slightly behind the chin to the animal's upper thorax. Overlying connective tissue was cut, and the underlying muscles separated along their central line to expose the trachea. After separation of the trachea and oesophagus, an opening was cut in the trachea and a tracheal cannula (a short section of Intramedic PE 240 polyethylene tubing with a tapered end) was inserted and tied in place.

Drugs were introduced into the animal *via* a cannula inserted into the femoral vein. An incision was made to expose the femoral vein, artery, and nerve. These were separated, and two ligatures positioned under the isolated femoral vein. The vein was tied by means of the lower (posterior) ligature. A small snip

was made in the vein anterior to the tie, and the venous cannula (a length of Intramedic PE 50 polyethylene tubing with one end drawn out and tapered, and the other attached to a saline-filled 1 ml syringe via a blunted 23 G needle) inserted into the vein and gently pushed down the vessel a distance of 1 or 2 cm. The anterior ligature was tied, being careful not to occlude the cannula. The latter was secured in position by a ligature sewn through the animal's leg, or alternatively, by means of a few thumb tacks on the dissecting board.

The rat's arterial blood pressure was continuously monitored by means of a cannula inserted in one of the carotid arteries. The arterial cannula (a length of Intramedic PE 50 polyethylene tubing with its free end tapered) was connected to an E and M transducer (Model P-100-A) via a blunt-ended size 23 G needle. The transducer was filled completely with Heparinized saline (approximately 1000 units per ml) via an attached 10 ml syringe. The cannula was filled with commercially supplied Heparin solution (10,000 units per ml). The transducer was connected to an amplifier and pen recorder (E and M Physiograph Model DMP-4A), and calibrated.

Either of the rat's two carotid arteries was selected and separated from surrounding connective tissue and the vagus and cervical sympathetic nerves for a distance of approximately 2 cm. The artery was tied off as far anteriorally as possible, and

posteriorly clamped with a bulldog clip. A ligature was inserted under the carotid, and a small snip made in the artery as close as possible to the anterior tie. The carotid cannula was inserted and tied securely in front of the bulldog clip, which was then removed. The circuit to the transducer was opened, and the mean arterial blood pressure recorded.

The compounds to be tested were dissolved in saline immediately prior to use, and introduced *via* the femoral venous cannula using a 1 ml disposable syringe fitted with blunted 23 G needles. After each injection, the drug was washed into the animal with 0.2 ml normal saline.

Experiment to Determine Protection of Aminohydroxamic Acids Against DFP Poisoning in Mice

Female, Alas strain mice of weight range 18 to 22 g were used.

DFP was transferred to a weighed sample vial in a fume hood. The vial and contents were reweighed, and adequate normal saline added so that 0.1 ml of the resulting solution contained 5 mg/kg when administered ip to a 20 g mouse.

Each amino-ester salt or aminohydroxamic acid hydrochloride was dissolved in normal saline so that 0.2 ml of the resulting solution given sc was equivalent to a dose of 200 mg/kg. Alternate

routes were used for injections of the amino-esters and hydroxamates, and the DFP, to minimize direct interaction between compounds.

Groups of three mice were injected sc with the desired dose of compound under investigation, followed 30 minutes later by an ip injection of DFP. Three mice, injected with normal saline instead of test compounds, were used as controls.

Each of the compounds under investigation was injected sc into one mouse at a dose level of 200 mg/kg, and after 8 hours the general condition of the animal noted.

2) PHARMACOLOGICAL EVALUATION OF ARYL- AND ARYLALKYL-AMINO-ESTER SALTS AND ARYLALKYLAMINOHYDROXAMIC ACID HYDROCHLORIDES

Preliminary Hypotensive Screen on Anaesthetized Rats

All compounds synthesized in this study were evaluated for their effect on the arterial blood pressure of normotensive anaesthetized rats. Each compound was tested at least twice in separate animals. The data obtained is shown in Tables 1 and 2.

TABLE 1

EFFECT OF 3-ARYLALKYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDES ON ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION min
68	1	3	-	-
	2	3	-	-
	10	1	-	-
	25	1	5	7
69	1	2	-	-
	2	2	-	-
	4	2	-	-
71	1	2	5	1.5
	2	2	10	2.5
	4	2	13	6.5
72	1	3	2	0.5
	2	3	4	0.75
	4	3	8	1
73	1	2	5	0.25
	2	2	7	0.25
	4	2	10	0.5
74	1	3	4	0.5
	2	3	9	4.5
	4	3	12	5
75	1	2	-	-
	2	2	-	-
	4	2	-	-
76	1	2	4	2
	2	2	7	4
	4	1	8	8
78	1	2	9	0.5
	2	2	15	0.5
	4	2	17	0.5

TABLE 1 (Cont'd)

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION min
82	1	2	-	-
	2	2	-	-
	4	2	-	-
86	1	2	-	-
	2	2	-	-
	4	1	-	-

N.B. In Table 1, a dash denotes inactivity of a compound at the dose level shown.

TABLE 2

EFFECT OF METHYL 3-ARYL- AND 3-ARYLALKYL-AMINO-PROPIONATE SALTS ON ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION min
45	0.5	2	-	-
	1	2	-	-
	2	2	-	-

TABLE 2 (Cont'd)

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION min
46	0.5	2	-	-
	1	2	-	-
	2	2	-	-
47	0.5	3	-	-
	1	3	-	-
	2	3	-	-
48	1	2	13	0.5
	2	2	19	1
	4	2	24	3
49	1	3	21	0.5
	2	3	26	1
	4	3	30	1.5
50	0.5	1	14	0.5
	1	3	15	0.5
	2	3	19	1.0
	4	2	25	1.0
51	1	3	28	0.5
	2	3	30	0.5
	4	2	14	1
52	1	2	-	-
	2	2	-	-
	4	2	-	-
53	1	2	-	-
	2	2	-	-
	4	2	-	-
54	1	2	-	-
	2	2	-	-
	4	2	-	-

TABLE 2 (Cont'd)

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION min
55	1	2	-	-
	2	2	-	-
	4	2	-	-
56	1	2	-	-
	2	2	-	-
	4	2	-	-
57	1	2	-	-
	2	2	-	-
	4	2	-	-
58	1	2	9	0.25
	2	2	37	1
	4	2	63	0.25
59	1	2	-	-
	2	2	-	-
	4	2	-	-
60	1	2	-	-
	2	2	-	-
	4	2	-	-
61	1	2	-	-
	2	2	-	-
	4	2	-	-
62	1	2	4	0.25
	2	2	15	0.25
	4	2	29	0.50
63	1	2	19	0.5
	2	2	26	1.5
	4	2	31	1.5

TABLE 2 (Cont'd)

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION min
64	1	2	-	-
	2	2	-	-
	4	2	-	-
65	1	1	4	0.25
	2	2	15	0.25
	5	2	45	0.50
	25	1	50	1
66	1	2	-	-
	2	2	-	-
	4	2	-	-

N.B. In Table 2, a dash denotes inactivity at the dose level shown.

Effect on Anaesthetized Rat Blood Pressure after Hyoscine Administration

Each active compound was administered intravenously to at least two hyoscine-treated rats to determine if the compound's hypotensive action was muscarinic in nature. ACh (0.05, 0.1, 0.2 μg) was given, followed by a suitable dose of the active compound. Hyoscine hydrobromide (1 mg) was then given, and the procedure repeated.

Only one compound, N,N-Di-(2-carbomethoxyethyl)phenethylamine hydrochloride (65) exhibited muscarinic properties by loss of activity after hyoscine administration.

Effect of Nicotine and Norepinephrine on the Blood Pressure of Rats Treated with Active Non-Muscarinic Compounds

All non-muscarinic active compounds were screened in the following manner. The anaesthetized rat was given small intravenous doses (generally, 20 to 40 μg) of nicotine, producing a rise in arterial blood pressure. Three doses of norepinephrine (0.05, 0.1 and 0.2 μg) were then administered. The rats were injected with the test compound (generally, 5, 10, or 25 mg/kg) and the administration of nicotine was repeated. An additional dose of test compound was then given, and administration of norepinephrine repeated.

It was found that in several cases the nicotine-induced rise in arterial blood pressure was completely blocked (but returned after a period of time), while in other cases only a partial block was observed. The response to noradrenaline was undiminished in all cases. In a few cases, both nicotine and noradrenaline responses remained undiminished after administration of the test compound

The results of this screening are presented in Tables 3 and 4.

TABLE 3

EFFECT OF NICOTINE ON THE BLOOD PRESSURE OF ANAESTHETIZED RATS TREATED WITH METHYL ARYLALKYLPROPIONATES

COMPOUND NUMBER IN TEXT	DOSE OF COMPOUND mg/kg	DOSE OF NICOTINE μg	RESPONSE TO NICOTINE AFTER GIVING COMPOUND	MECHANISM OF ACTION
45	25	30	+ -	UNDEFINED
48	10	20	-	GANGLIONIC BLOCKADE
	25	20	-	
49	25	20	-	GANGLIONIC BLOCKADE
50	25	20	-	GANGLIONIC BLOCKADE
51	25	20	-	GANGLIONIC BLOCKADE
56	50	20	-	GANGLIONIC BLOCKADE
58	10	20	-	GANGLIONIC BLOCKADE
62	4	40	+ -	UNDEFINED
63	4	40	+ -	UNDEFINED

N.B. In Table 3: + - denotes diminished response
+ denotes undiminished response
- denotes absence of response

to nicotine after administration of test compound.

TABLE 4

EFFECT OF NICOTINE ON THE BLOOD PRESSURE OF ANAESTHETIZED RATS TREATED WITH ARYLALKYLAMINOHYDROXAMIC ACID HYDROCHLORIDES

COMPOUND NUMBER IN TEXT	DOSE OF COMPOUND mg/kg	DOSE OF NICOTINE µg	RESPONSE TO NICOTINE AFTER GIVING COMPOUND	MECHANISM OF ACTION
71	25	20	+ -	UNDEFINED
72	25	20	-	GANGLIONIC BLOCKADE
73	50	20	+ -	UNDEFINED
74	10	20	+ -	UNDEFINED
76	50	20	+	UNDEFINED
78	10	40	-	GANGLIONIC BLOCKADE

N.B. In Table 4: + - denotes diminished response
+ denotes undiminished response
- denotes absence of response,

to nicotine after administration of test compound.

Screening in Mice of Methyl Aryl- and Arylalkylaminopropionates and Arylalkylaminopropionohydroxamic Acids as Protective Agents Against Lethal Organophosphate Poisoning

All amino-esters and aminohydroxamates synthesized in the

present study were screened for their potential protection against DFP (5 mg/kg ip) in female Alas strain mice.

The DFP dosage (5 mg/kg) (approximately twice the LD_{50}) was chosen in accordance with an LD_{50} determination (Selley, 1971).

TABLE 5

DETERMINATION OF THE LD_{50} OF DFP IN FEMALE ALAS STRAIN MICE

DOSE mg/kg	NUMBER DEAD	NUMBER ALIVE
2.0	2	8
2.5	6	4
3.0	9	1

A calculation of the LD_{50} of DFP based upon the data in Table 5 has given (Selley, 1971) the LD_{50} as 2.38 (limits 2.09 to 2.71) mg/kg.

Each compound was administered sc (200 mg/kg) to groups of four mice. DFP was administered ip (5 mg/kg) to three of the four mice in each group 30 minutes after injection of the test compound. The remaining mouse in each group was not injected with DFP, but was observed 8 hours after compound administration for

general toxic reactions. A group of three mice was used as a control, each mouse receiving only 0.2 ml normal saline before DFP administration.

All mice receiving DFP died within a few minutes.

Those mice receiving only the test compound appeared normal after 8 hours, although initially exhibiting signs of excitation and disorientation.

CHAPTER 5

DISCUSSION OF THE PHARMACOLOGY OF ARYLALKYLAMINOHYDROXAMIC ACIDS AND METHYL ARYL- AND ARYLALKYL-AMINO-ESTERS

1) EFFECT OF 3-ARYLALKYLAMINOPROPIONOHYDROXAMIC HYDROCHLORIDES ON THE ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

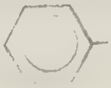
The pharmacological results obtained with the aminohydroxamic acids synthesized in this study are presented in Tables 1 and 4.

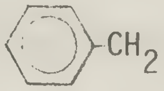
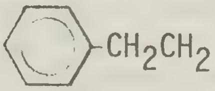
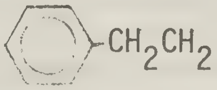
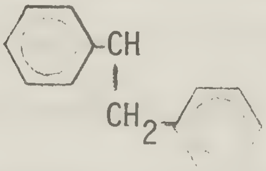
The results will now be discussed with reference to structural features. It is convenient to divide the various hydroxamates into groups on a basis of α- and β-branching.

The following 3-arylalkylaminopropionohydroxamic acids of general structure (67) where R^3 and R^4 are hydrogen were examined:

$$R^1R^2\overset{+}{N}HCHR^3CHR^4CONHOH\ Cl^-$$

(67)

COMPOUND	R^1	R^2	R^3	R^4
68	 $-CH_2$	H	H	H
71	$-CH_2CH_2$	H	H	H
74	$-CH_2CH_2CH_2$	H	H	H

COMPOUND	R^1	R^2	R^3	R^4
75		CH_3	H	H
78			H	H
82		H	H	H

Little has been reported regarding the hypotensive effects of compounds of general structure (67) where R^1 and possibly R^2 are arylalkyl groups.

Coutts et al (1969) has reported the pharmacological evaluation of one of the above compounds, namely, 3-benzylaminopropionohydroxamic acid hydrochloride (68). It was reported that the latter compound (at 25 mg/kg) gave a 45 mm fall (over one hour in duration) in the arterial blood pressure of an anaesthetized cat. In contrast, the same compound did not give similar results in the present study, but was found inactive in anaesthetized rats at doses up to 25 mg/kg.

Introduction of an additional methylene group into the amino chain of compound (68) to give 3-phenethylaminopropionohydroxamic acid hydrochloride (71), resulted in a great increase in hypotensive activity.

Substitution of a methyl group at the secondary amino nitrogen of 3-benzylaminopropionohydroxamic acid hydrochloride (68) gives 3-(N-methylbenzylamino)propionohydroxamic acid hydrochloride (75), prepared and found inactive.

Incorporation of an additional phenethyl group into compound (71) gave 3-(di-2-phenylethylamino)propionohydroxamic acid hydrochloride (78), a compound with a marked but brief hypotensive effect. (The N-benzylphenethylamino analogue of the latter compound could not be obtained for pharmacological comparison.)

Substitution of an additional benzyl group at the methylene function of 3-benzylaminopropionohydroxamic acid hydrochloride (68) gives 1,2-diphenylethylaminopropionohydroxamic acid hydrochloride (82), a compound found inactive in the present study.

The only bis hydroxamic acid successfully synthesized in this investigation, namely, N-phenethyliminodipropionohydroxamic acid hydrochloride (86) lacked hypotensive activity. (Unfortunately, the benzylamine analogue was not available for testing.) It may be that alteration of an active secondary aminohydroxamate such as 3-phenethylaminopropionohydroxamic acid hydrochloride (71) to a tertiary amino bis type structure results in loss of hypotensive activity.

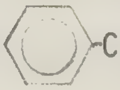
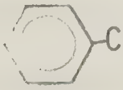
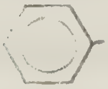
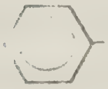
Due to difficulties in recrystallization, several unbranched aminohydroxamates could not be synthesized, and the structure-activity relationships which can be drawn from the

available data are tentative.

3-Arylalkylaminopropionohydroxamic acid hydrochlorides of general structure (67) wherein R^4 is methyl and R^3 is hydrogen were also evaluated in the present study;

$$R^1R^2\overset{+}{N}HCHR^3CHR^4CONHOH\ Cl^-$$

(67)

COMPOUND	R^1	R^2	R^3	R^4
69	 $-CH_2$	H	H	CH_3
72	 $-CH_2CH_2$	H	H	CH_3
74	 $-CH_2CH_2CH_2$	H	H	CH_3
76	 $-CH_2$	CH_3	H	CH_3

Substitution of an α-methyl group on the inactive 3-benzylaminopropionohydroxamic acid hydrochloride (68) produces 2-methyl-3-benzylaminopropionohydroxamic acid hydrochloride (69), a compound synthesized and shown inactive.

The hypotensive action of 2-methyl-3-phenethylaminopropionohydroxamic acid hydrochloride (72), the α-methyl derivative of 3-phenethylaminopropionohydroxamic acid hydrochloride (71), has been previously evaluated by Coutts et al (1971). 5 mg/kg and

10 mg/kg of the former compound produced 18% and 35% falls, respectively, in the arterial blood pressure of the anaesthetized cat. In the present study, the same compound tested in rats at 4 mg/kg produced an 8% fall in arterial blood pressure of much shorter duration than that reported by Coutts and his group. It appears that α-methyl substitution on 3-phenethylaminopropionohydroxamic acid hydrochloride (71) produces a compound with a less potent and shorter hypotensive action.

2-Methyl-3-phenylpropylaminopropionohydroxamic acid hydrochloride (74), the higher methylene homologue of 2-methyl-3-phenethylaminopropionohydroxamic acid hydrochloride (72), exhibited moderately high hypotensive activity at 4 mg/kg. Thus it was observed that an increase in alkylamino chain length (i.e., progression from benzylamino- to phenylpropylaminohydroxamates) produced an increase in degree and duration of hypotensive action.

2-Methyl-3-(N-methylbenzylamino)propionohydroxamic acid hydrochloride (76), the N-methyl derivative of 2-methyl-3-benzylaminopropionohydroxamic acid hydrochloride (69), was active, in contrast to the latter inactive compound. (In contrast, as previously mentioned, N-methyl substitution on the unbranched analogue does not give an active compound.)

Only one hydroxamate of general formula (67) where R^3 is methyl and R^4 is hydrogen was synthesized, namely, 3-methyl-3-phenethylaminopropionohydroxamic acid hydrochloride (73). This

compound exhibited hypotensive action of moderately high intensity but brief duration.

It should be noted that no arylaminohydroxamates were synthesized in the present study. Preliminary pharmacological evaluation showed several arylamino-esters (various anilinopropionates) devoid of hypotensive activity, and therefore, the corresponding aminohydroxamic acid hydrochlorides were not prepared.

As outlined in Table 4, active hydroxamates in the present investigation either partially or completely blocked the hypertensive effect of low doses of nicotine, suggesting that these compounds act partially or completely *via* ganglion blockade. Recovery of response to nicotine was demonstrable in all cases.

None of the active hydroxamic acids blocked the pressor effect of norepinephrine, ruling out a mechanism of action involving α-adrenergic receptor blockade.

Hydroxamate-induced hypotension in anaesthetized rats was not blocked by the administration of hyoscine, whereas hypotension produced by ACh administration was eliminated. These observations suggest that these compounds do not act muscarinically.

In conclusion, the present investigation suggests that the nature of the amino moiety (*i.e.*, R^1 and R^2) is critical for hypotensive action in amino-hydroxamates, but due to the limited number of compounds available for evaluation, it is unwise to generalize about the nature of R^1 and R^2.

Alkyl substitution at the α- or β-carbon of the amino-hydroxamates also affected hypotensive activity, but again, no generalizations are possible due to the small number of such compounds synthesized.

The hydroxamic acid hydrochlorides prepared in the present study had short durations of action.

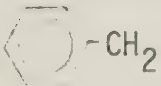
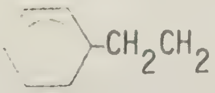
2) EFFECT OF METHYL 3-ARYL AND 3-ARYLALKYL-AMINOPROPIONATES ON THE ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

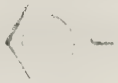
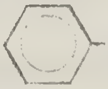
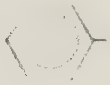
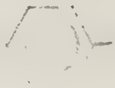
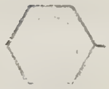
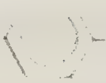
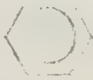
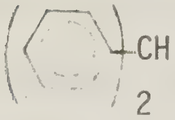
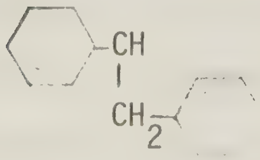
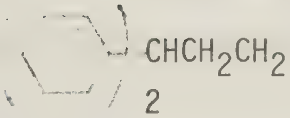
The pharmacological data for the amino-esters synthesized in this study are presented in Tables 2 and 3.

The following methyl 3-aryl and 3-arylalkylaminopropionates of general structure (87) where R^3 and R^4 are hydrogen were investigated:

$$R^1R^2\overset{+}{N}HCHR^3CHR^4COOCH_3\ X^-$$

(87)

COMPOUND	R^1	R^2	R^3	R^4
45		H	H	H
48		H	H	H

COMPOUND	R^1	R^2	R^3	R^4
52		H	H	H
55	 $-CH_2$	CH_3	H	H
58	 $-CH_2CH_2$	 $-CH_2CH_2$	H	H
59	 $-CH_2CH_2$	$-CH_2$	H	H
60	 $-CH_2$	 $-CH_2$	H	H
61		H	H	H
62		H	H	H
63		H	H	H

Methyl 3-benzylaminopropionate hydrobromide (45) was found to be inactive at 2 mg/kg in the present study. It has been reported (Coutts _et al_, 1969) that this compound (at 25 mg/kg) produced a 25% fall in the arterial blood pressure of an anaesthetized cat.

Methyl 3-phenethylaminopropionate hydrobromide (48),

the higher methylene homologue of compound (45), produced a 24% fall in blood pressure at a dose of 4 mg/kg.

Elimination of the methylene moiety of the amino component of unbranched amino-ester resulted in complete loss of hypotensive activity. This is illustrated by the inactive methyl 3-anilinopropionate hydrobromide (52).

Methyl substitution on the secondary amino nitrogen of methyl 3-benzylaminopropionate hydrobromide (45), inactive as previously mentioned, gives the equally inactive methyl 3-(N-methylbenzylamino)propionate hydrobromide (55).

Several other arylalkylamino configurations were employed in an effort to obtain hypotensive amino-esters. Methyl 3-(di-2-phenylethylamino)propionate hydrobromide (58) had the most potent hypotensive action of any amino-ester in the present study.

A slight alteration of the latter structure gives methyl 3-(N-benzylphenethylamino)propionate hydrobromide (59) lacking hypotensive activity.

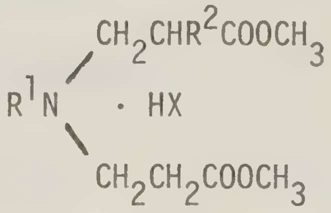
A related compound, methyl 3-dibenzylaminopropionate hydrochloride (60), was also inactive. This is interesting because of this compound's close relationship to the adrenergic blocking agent N-(2-chloroethyl)dibenzylamine hydrochloride (Dibenamine).

Methyl 3-(diphenylmethylamino)propionate hydrobromide (61) was inactive, but in contrast, the higher methylene homologue of the latter compound, methyl 3-(1,2-diphenylethylamino)propionate

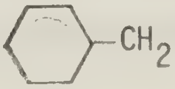
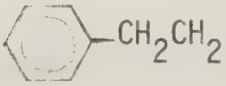
hydrobromide (62), exhibited a moderate but brief hypotensive action.

Methyl 3,3-diphenylpropylaminopropionate hydrobromide (63) displayed similar brief and moderate activity.

The following amino-di-esters of general structure (88) where R^2 is hydrogen were synthesized:



(88)

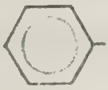
COMPOUND	R^1	R^2
64		H
65		H

Of the unbranched amino-di-esters synthesized, only N,N-Di-(2-carbomethoxyethyl) phenethylamine (65) displayed hypotensive activity, which was marked but brief.

Amino-esters of general formula (87) where R^4 is methyl were also prepared:

$$R^1R^2\overset{+}{N}HCHR^3CHR^4COOCH_3\ X^-$$

(87)

COMPOUND	R^1	R^2	R^3	R^4
46	 -CH_2	H	H	CH_3
49	-CH_2CH_2	H	H	CH_3
51	-$CH_2CH_2CH_2$	H	H	CH_3
56	-CH_2	CH_3	H	CH_3
53	-	H	H	CH_3

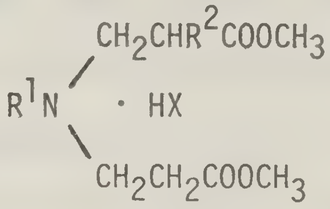
The α-substituted derivative of the inactive compound methyl 3-benzylaminopropionate hydrobromide (45), namely, methyl 2-methyl-3-benzylaminopropionate hydrobromide (46), was also inactive.

The higher methylene homologue of the latter compound, methyl 2-methyl-3-phenethylaminopropionate hydrobromide (49) was active, as previously reported by Coutts et al (1971). It is interesting that this branched amino-ester was slightly more active than its unbranched relative, methyl 3-phenethylaminopropionate hydrobromide (48).

Methyl 2-methyl-3-anilinopropionate hydrobromide (53) was inactive, as were other arylamino-esters synthesized in this study.

Substitution at carbon-2 of inactive methyl 3-(N-methylbenzylamino)propionate hydrobromide (55), gives methyl 2-methyl-3-(N-methylbenzylamino)propionate hydrobromide (56), also inactive.

An α-branched amino-di-ester of general formula (88)



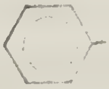
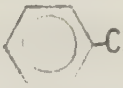
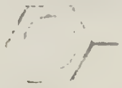
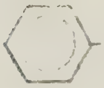
(88)

where R^2 is methyl and R^1 was benzyl was synthesized. N-(2-carbomethoxyethyl)-N-(2-carbomethoxypropyl)benzylamine hydrochloride (66) was inactive.

Several other amino-esters of general formula (87) where R^3 is methyl were prepared to complete the present series:

$$R^1R^2\overset{+}{N}HCHR^3CHR^4COOCH_3\ X^-$$

(87)

COMPOUND	R^1	R^2	R^3	R^4
47	 $-CH_2$	H	CH_3	H
50	 $-CH_2CH_2$	H	CH_3	H
54		H	CH_3	H
57	 $-CH_2$	CH_3	CH_3	H

The above compounds, with the exception of methyl 3-methyl-3-phenethylaminopropionate hydrobromide (50), were found inactive as hypotensive agents. The latter compound was among the more active of the amino-esters synthesized during the present study. Thus it would appear that β-methyl substitution of an inactive unbranched ester, such as methyl 3-benzylaminopropionate hydrobromide (45), does not confer hypotensive activity.

In conclusion, the amino moiety of the amino-ester (i.e., R^1 and R^2) is most important in determining the degree or presence of hypotensive activity in the amino-esters of this study. Methyl substitution at carbon-2 or carbon-3 may alter activity, but does not confer it.

The mechanisms of action of the active amino-esters were investigated, the technique used being identical to that employed with the amino-hydroxamates.

Many of the active amino-esters partially or completely blocked the hypertensive effect of low doses of nicotine for a short period of time, after which response to nicotine was recovered. Those amino-esters which completely blocked the hypertensive effects of nicotine most likely act via ganglion blockade. The clarification of modes of action of those compounds which partially blocked nicotine was not attempted.

None of the amino-esters diminished the response to noradrenaline.

Only one amino-ester, N,N-Di-(2-carbomethoxyethyl)-phenethylamine hydrochloride (65) displayed muscarinic action. This is an interesting observation, since it suggests that while the mono-esters act via ganglion blockade, di-esters may act muscarinically.

The durations of action of the amino-esters synthesized in this study were short, precluding their use as anti-hypertensives.

3) EFFECTIVENESS OF 3-ARYLALKYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDES AND METHYL 3-ARYL AND 3-ARYLALKYL-AMINO-ESTERS IN PROTECTING AGAINST LETHAL ORGANOPHOSPHATE POISONING

All the amino-esters and aminohydroxamates synthesized in this study were screened for potential in protection against organophosphate poisoning. None of the compounds were found effective in this role.

In the accompanying toxicity screen, none of the compounds in this investigation were lethal to single mice at doses of 200 mg/kg, but many of the animals showed some signs of disorientation and excitation.

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